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PATHOLOGY

Also the Official Organ of the American Society for Experimental Pathology

AMERICAN MEDICAL ASSOCIATION PUBLICATION

TABLE OF CONTENTS

VOLUME 68 SEPTEMBER 1959 NUMBER 3

ORIGINAL ARTICLES

REGULAR DEPARTMENTS

A. M. A.
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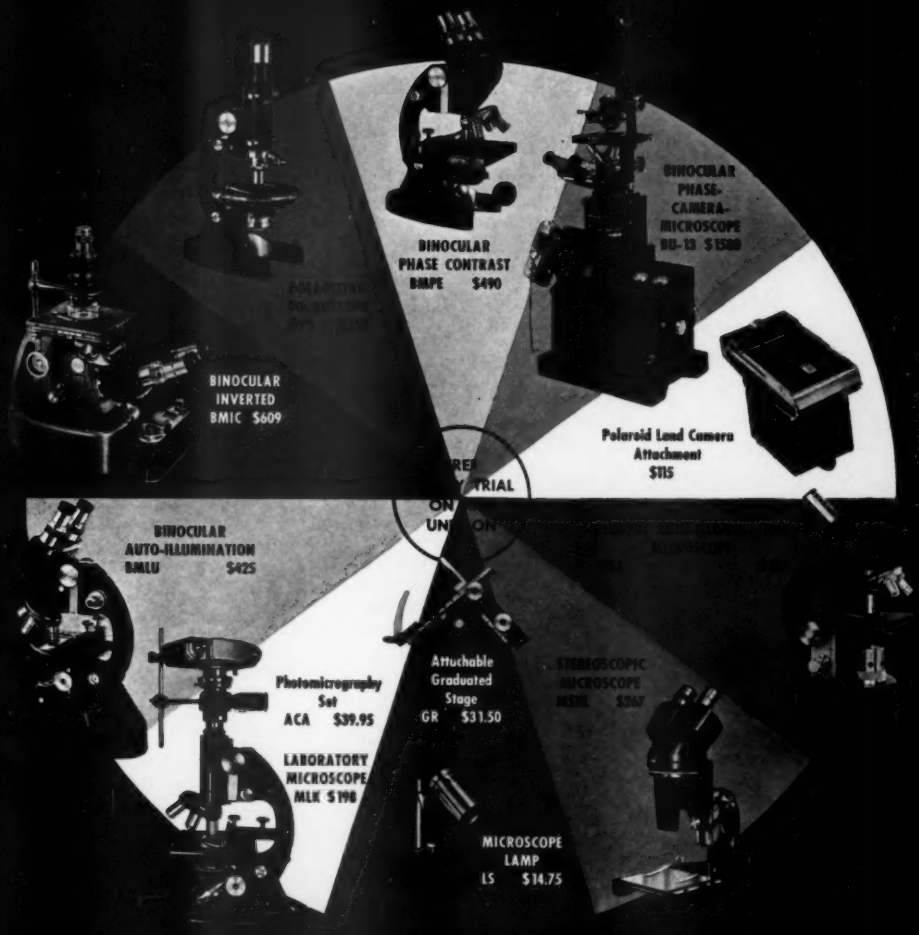

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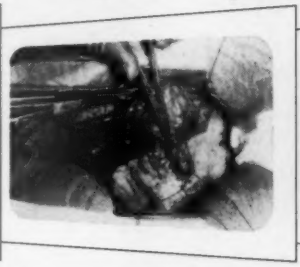

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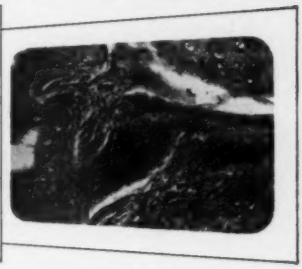

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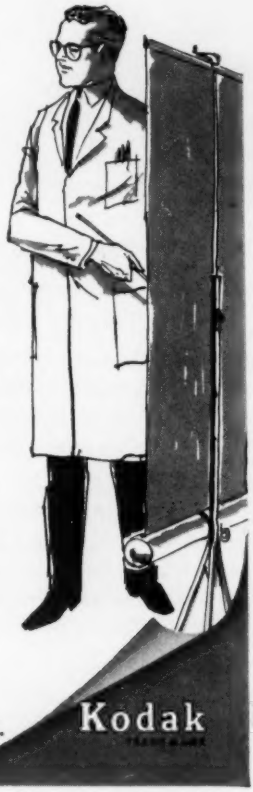

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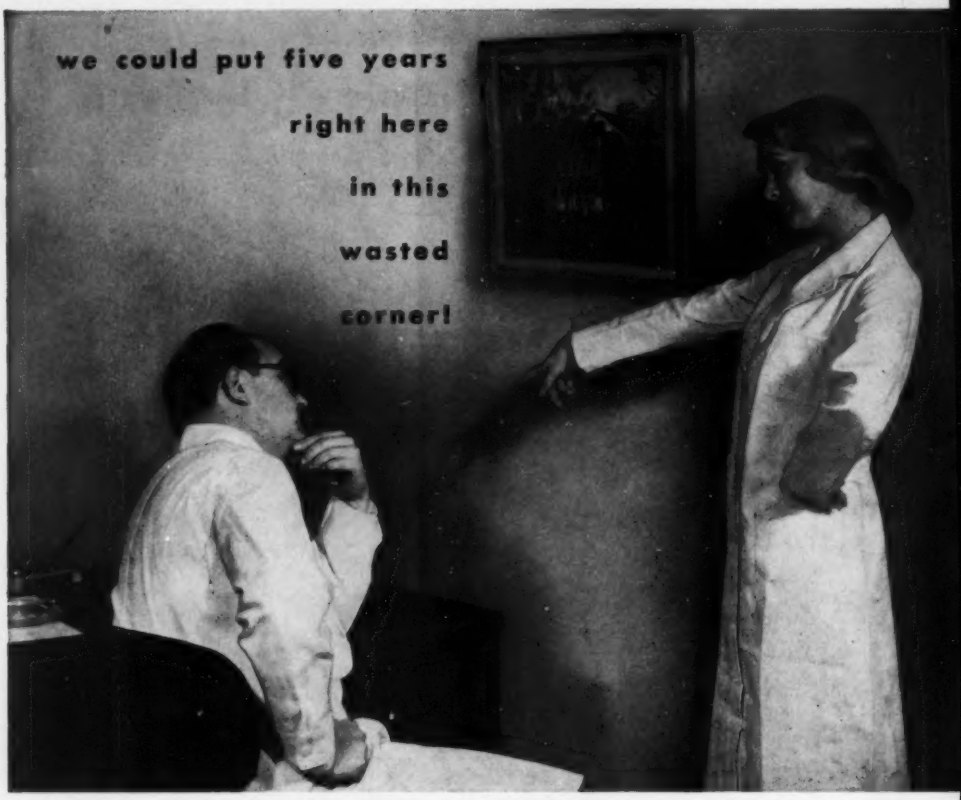

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Incidence, Distribution, and Enzymatic Activity of Carcinoma of the Prostate Gland

JAMES BUTLER, M.D.; HERBERT BRAUNSTEIN, M.D.; DAVID G. FREIMAN, M.D., and EDWARD A. GALL, M.D., Cincinnati

An appreciation of the comparatively high incidence of occult carcinoma of the prostate gland in elderly men has stemmed from the observations of Muir,[1] Rich,[2] and Moore.[3] In Rich's cases, the lesions were found for the most part to be located beneath the capsule in both lateral and posterior lobes, while in Moore's investigation almost three-fourths of the neoplasms were located in the posterior aspect of the gland. The predilection for posterior localization was substantiated by Gaynor.[4] Kahler,[5] on the other hand, found more lesions originating in the lateral lobes. An indication of multicentricity of origin was provided by Moore,[3] who observed 68 neoplastic foci in 52 prostate glands containing small carcinomas. Gaynor[4] and Kahler[5] also described multiple sites of origin.

The present study was designed to investigate by means of large tissue sections the frequency with which occult carcinoma arises simultaneously in multiple portions of the prostate gland and to plot the distribution of these lesions. An ancillary purpose was the investigation of the enzymatic activity of neoplastic and non-neoplastic prostatic epithelium by histochemical means.

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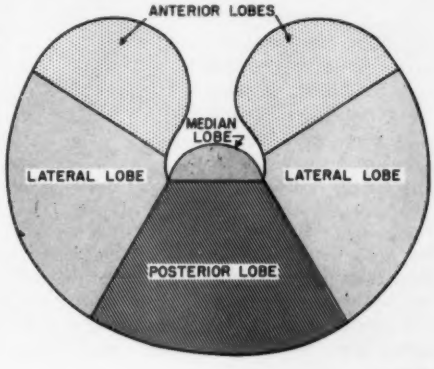
This study was supported in part by a research grant [CS-9076(C4)] from the National Institutes of Health, U. S. Public Health Service.

Materials and Methods

Two hundred twenty prostate glands were secured at necropsy from men over 50 years of age at the Cincinnati General Hospital. No selection was made except for the exclusion of all cases in which a diagnosis of prostatic carcinoma had been made before death. Seven cases in which carcinoma was recognized macroscopically at necropsy were included in the study, as noted below.

One or more coronal sections were taken through the entire prostate gland at its midportion, in a plane perpendicular to the prostatic portion of the urethra. A single section was usually procured in each case. This section included major portions of posterior, lateral, and anterior "lobes," as indicated

Fig. 1.—Outline of the anatomical divisions of the prostate at the level usually sectioned.



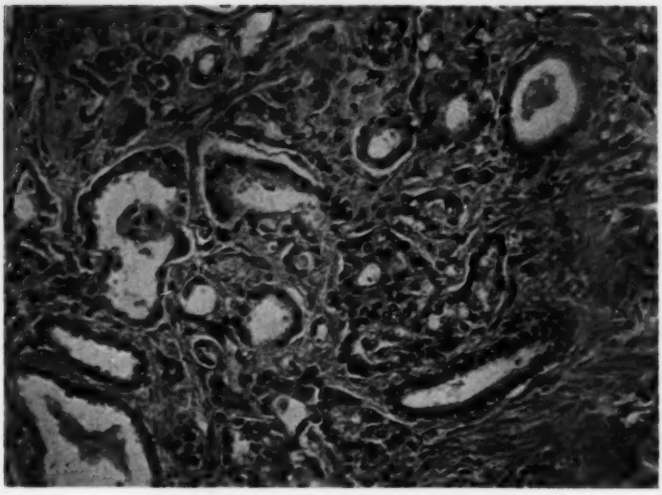


Fig. 2.—Focus of carcinoma illustrating invasion of the stroma. Hematoxylin and eosin stain; × 500.

in Figure 1. Most of the sections passed through the region of the verumontanum. The tissue block thus secured was fixed in 10% buffered formalin, processed, embedded in paraffin, and sectioned at 6μ to 8μ intact. One section from each block was stained with hematoxylin and eosin and surveyed for foci of adenocarcinoma. The locations of the neoplasms thus found were then marked for mapping of distribution.

Criteria for the microscopic recognition of carcinoma were as follows:

1. Evidence of invasion of stroma, blood vessels, or perineural lymphatic channels (Figs. 2, 3)
2. Neoplastic alteration of the glandular pattern
   (*a*) Sheets and masses of cells in place of well-formed acini
   (*b*) Multiple small glands, frequently without lumens, occasionally without separation by fibromuscular bands (Fig. 3)
   (*c*) Secondary acinus formation (Fig. 3)
   (*d*) Marked papillary infolding accompanied by cytologic atypism
3. Cytologic alteration
   (*a*) Cellular anaplasia
   (*b*) Mitotic activity
   (*c*) Loss of mucin secretion

In 27 instances histochemical investigation of enzyme activity was carried out. Eight cases manifesting carcinoma in the large tissue sections were included in this group, but only six of these proved to contain neoplasm in the tissues selected for histochemical examination. The remaining 19 cases contained neoplasm in neither the large tissue sections nor the samples selected for special studies. The enzymes tested were nonspecific esterase, acid phosphatase, 5-nucleotidase, and alkaline phosphatase. The techniques utilized have been described in a previous report.[6]

Results

A. Large Tissue Sections.—The incidence of occult carcinomas is illustrated in Table 1. Among the 220 glands examined, 7 carcinomas, unsuspected clinically, were recognized grossly by the prosectors. These are indicated separately in the tabulation. Among the 213 remaining glands in which no gross evidence of neoplasm was detected, 64 were found to contain one or more foci of carcinoma when surveyed microscopically, an incidence of 30.0%. If the seven grossly recognized lesions are included in the tabulation, the incidence of clinically occult carcinoma in this series is 32.2%. It should be reemphasized that the examination of a single transverse section was the rule; no effort was made to examine several sections.

TABLE 1.—*Incidence of Occult Carcinoma of the Prostate*

No. of prostates examined	220
No. of carcinomas recognized by gross inspection	7
No with carcinoma unrecognized by gross inspection	213
No. containing occult carcinoma	64 (30.0%)
Incidence of clinically occult carcinoma	32.2%

TABLE 2.—*Multicentric Carcinoma of the Prostate*

No. of cases	71
Single focus of neoplasm	40 (56.4%)
Multicentric foci	26 (36.6%)
Whole gland (diffuse invasion)	5 (7.0%)
Neoplastic Foci	
Two	15
Three	7
Four	2
Five	2
Lobar Distribution	
Single lobe	2
Multiple lobes, unilateral	7
Multiple lobes, bilateral	17
Variation in histologic pattern	13

Table 2 illustrates the distribution of the lesions in the 71 glands containing neoplasms. In 40 (56.4%) only a single neoplastic lesion was detected; in 26 (36.6%) there were multiple lesions (Figs. 2, 3, 4), and in 5 (7.0%) the entire section (Fig. 5) was diffusely affected. It appears logical to assume that step or multiple sections would have increased to even higher proportions the yield of multicentric carcinomas. Also noteworthy are the high frequency with which three or more foci were noted in a

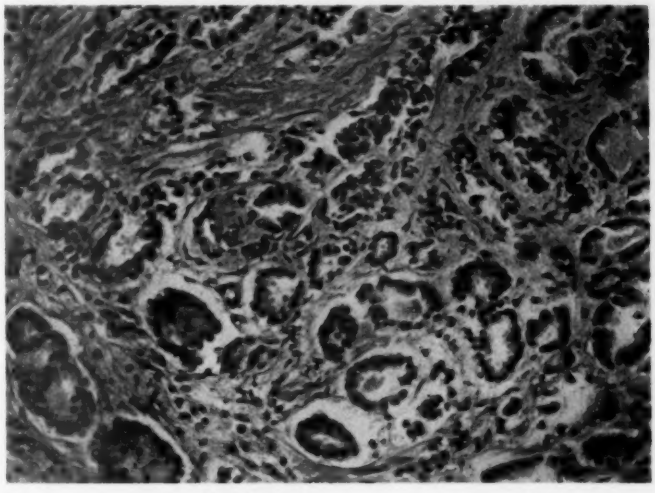


Fig. 3.—Focus of carcinoma from a different area of the same gland as that illustrated in Figure 2. There are many small acini, occasionally without lumens. Secondary acinus formation may be seen. Some foci are apparently within the lumens of vascular channels. Hematoxylin and eosin stain; × 500.

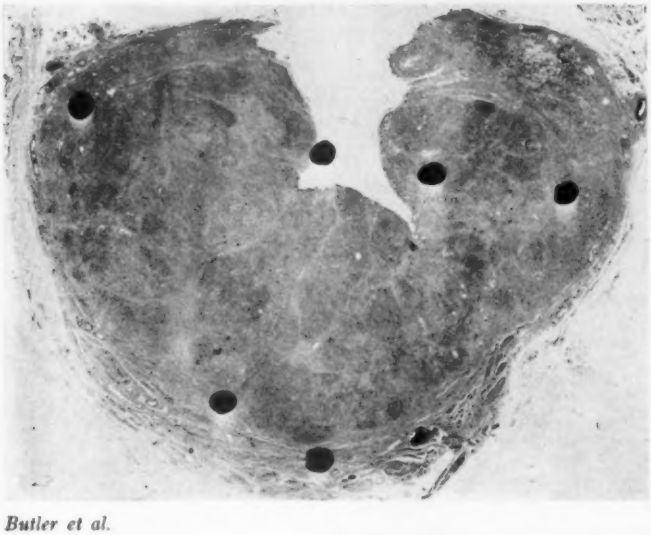


Fig. 4. — Low-power magnification of a large tissue section of a second prostate gland. Individual foci of carcinoma were found in the areas indicated by each ink dot. Hematoxylin and eosin stain; × 3.

Fig. 5. — Low-power magnification of a large tissue section of a third prostate gland. Carcinoma is present in all areas and invades the periprostatic tissue posteriorly. Hematoxylin and eosin stain; ×3.

single gland, and the observation of variation in histologic pattern within neoplastic foci in 13 instances.

Table 3 illustrates the localization of the lesions in 66 cases. The total is in excess of 100% because of the frequency of multiple lesions. It is noteworthy that the lateral "lobes" were more commonly affected in this series and that of all cases in which there was carcinoma there was lateral "lobe" involvement in 89.4% of cases and posterior "lobe" involvement in 33.3%. (If lesions appearing in a posterior position, but considered to arise in the lateral lobes, are included in the posterior group, these incidences become 66.7% and 56.4%, respectively.)

B. Histochemical Findings.—The histochemical findings are summarized in Table 4. One notes that cells may be mapped to a major extent by the pattern of their enzyme activity. No qualitative differences existed between normal and neoplastic glandular and stromal elements. Since the tissue was obtained at varying intervals post mortem, no attempt could be made to quantitate activity generally. In individual cases there appeared to be differences in the activity of normal and neoplastic acini, the latter showing somewhat lower degrees of activity, although in one specimen the carcinoma cells had more marked acid phosphatase and esterase activity. The degree of enzyme activity did not appear to vary with the degree of differentiation of the tumor, but this feature was frequently difficult to evaluate in the type of material (postmortem) available. The only unique discernible feature was the total disorganization of prostatic architecture in zones of neoplastic alteration, clearly revealed in sections prepared to demonstrate enzymes (Figs. 6 and 7).

TABLE 3.—*Lobar Location of Prostatic Carcinoma*

Total number of cases (excluding those affecting entire gland)	66
Anterior lobe	10 (15.3%)
Lateral lobe	21 (31.4%)
Anterolateral	18 (27.3%)
Midlateral	5 (7.6%)
Posterolateral	15 (22.7%)
Total lateral	59 (89.4%)
Median lobe	0
Posterior lobe	22 (33.3%)

TABLE 4.—*Distribution of Enzyme Activity in the Prostate as Determined Histochemically*

Enzyme	Alkaline Phosphatase	Acid Phosphatase	Nonspecific Esterase	5-Nucleotidase
Normal acini	0	+	+	0 *
Neoplastic acini	0	+	+	0 *
Smooth muscle	0	0	0	+
Fibrocytes	0	0	0	0
Capillary endothelium	+	0	0	0
Histiocytes	0	+	+	0
Neutrophils	+	0	0	0

* Nuclear staining upon prolonged incubation.

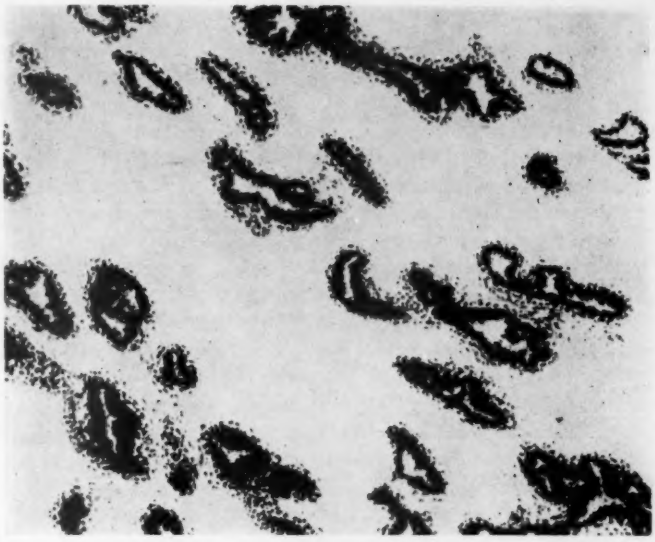


Fig. 6.—Section of normal prostate gland, illustrating the strong acid phosphatase reaction in acini. Normal glandular pattern is clearly manifest. Gomori technique; × 90.

Comment

A search of 17 standard reference works in urology, pathology, and neoplastic disease [7-23] indicates that all call attention to the great frequency of carcinoma of the prostate in elderly men. In 14 [7-11,13-15,18-23] of the 17 references the cancer is described as originating predominantly in the posterior lobe. Moore,[3] Rich [2] (erroneously), and Gaynor [4] were cited in support of this contention. In the remaining three references [12,16,17] there is no statement as to predilectivity of location. The occurrence of multicentricity in this type of neoplasm is never mentioned.

It is of interest to compare the observations made in the present series of cases with those recorded in similar studies.

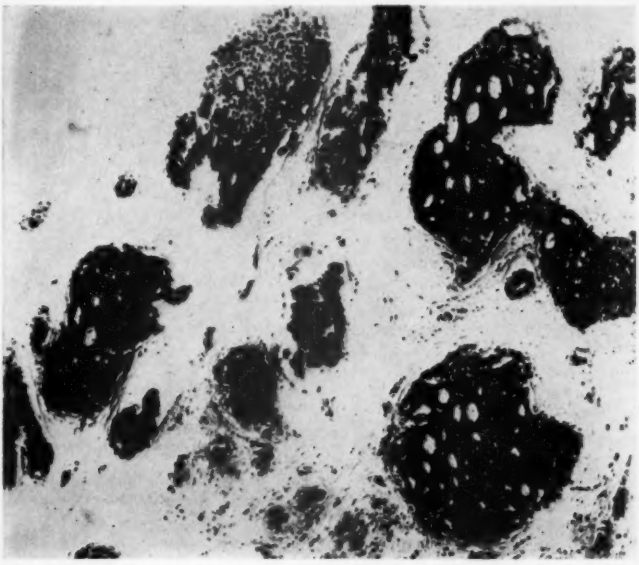


Fig. 7. — Section of prostate affected by carcinoma, illustrating disruption of normal architecture by proliferating neoplastic cells. Gomori technique; × 90.

TABLE 5.—*Reported Incidence of Occult Carcinoma of the Prostate in Men Over Fifty*

Series	No. of Prostates Examined	No. with Carcinomas	% Incidence
Butler & others	213 (220)	64 (71)	30.0 (32.2)
Muir[1]*	54	12	13
Rich[2]	292	41	14
Moore[3]	375	79	21
Kahler[5]†	--	--	17.3
Gaynor[4]	888	184	20.7
Edwards et al.[24]	150	28	18.7
Quinland[19]‡	188	34	18.0
Andrews[25]	99	16	16.2
Hudson et al.[20]§	250	37	14.8
Baron & Angrist[28]	50	23	46.0
Hirst & Bergman[27]‖	39	21	53.8
Franks[26]	180	69	37.2
Wilson & McGrath[21]¶	468	73	15.5

* Surgical specimens only, men over 60 years old.

† Figures not broken down; include cases previously recognized clinically.

‡ Both surgical specimens and necropsy cases; men over 40; 14 of 34 cases recognized preoperatively or before death.

§ Surgical biopsy only.

‖ Men 80 years old and more.

¶ Surgical specimens, diseased prostates; number recognized preoperatively not stated.

TABLE 6.—*Reported Incidence of Carcinoma of the Prostate with Multiple Sites*

Series	
Butler et al.	71 cases; 26 multifocal; 5 entire gland
Moore[3]	52 cases; 68 foci described
Kahler[5]	96 cases localized; 8 multicentric
Edwards et al.[24]	29 cases; 8 multicentric
Quinland[19]	"Often a commingling of different types of cellular arrangement in the same neoplasm"
Gaynor[4]	162 cases with single-lobe involvement; 34 with 2 or more foci; 10 cases with lesions in 2 lobes; 19 entire gland
Hudson et al.[20]	No figures but stated that lesions may be multicentric
Franks[26]	69 cases: 23 with more than one tumor nodule

Table 5 compares the incidence of occurrence. All figures listed relate to incidence in men over 50 years of age unless otherwise indicated in the footnotes. The high frequency of occult carcinoma of the prostate has been repeatedly emphasized in the past. Our own experience warrants no extended comment, save to indicate confirmation. Of interest is the very high incidence of carcinoma in this series, despite the use of a relatively simple survey method. Indeed, the yield of others using similar techniques (Kahler[5]; Edwards et al.[24]) compare reasonably well with those obtained by investigators utilizing more extensive step-section methods (Moore[3]; Andrews[25]). Our results (32.2%) do not differ markedly from comparable reports, indicating an incidence of 37.2% to 46.0% (Franks[26]; Hirst and Bergman[27]; Baron and Angrist[28]).

Table 6 compares the frequency of multicentricity in the present series with that described in other series where this phenomenon is either implied or mentioned. The appreciation of the fact that prostatic carcinoma may arise in multiple independent sites has in the past been alluded to only casually. It would appear to warrant greater emphasis in view of the frequency (7.7% to 43.7%) with which it has been noted. Among 66 examples of carcinoma in which the entire gland was not affected, there were encountered 107 separate sites of carcinoma. The frequency with which three and more foci were identified in a single gland is especially noteworthy and

TABLE 7.—*Reported Locations of Carcinoma of the Prostate*

	No.	Lateral, %	Posterior, %	Anterior, %	Other
Butler et al.	66	89.6	33.3	15.3	5 whole gland
Rich[2]	41	Subcapsular, both lateral and posterior			
Moore[3]	79	8.8	73.5	14.8	--
Kahler[5]	96	48	46	6	--
Gaynor[4]	184	9.8	60.6	29.1	0.5% median
Edward et al.[24]	41	34.2	51.2	14.6	87.8% subcapsular
Andrews[25]	20	40	60	--	--
Baron and Angrist[28]	23 (13)	69.2	30.8	--	10 cases not localizable
Franks[26]	69	Most posterior and posterolateral, only 2 anterior, 45 subcapsular			
Palomo[23]	276	?	75	--	--

may offer a clue in explanation of diffuse glandular involvement observed with such high frequency in cases of clinically overt prostatic cancer.

Table 7 summarizes the localization of neoplastic foci in the present group of cases and other surveys of like nature. The predilective localization of early carcinoma of the prostate shown by our group of cases is at variance with that of most other reports in the literature. In only two other groups of cases (Baron and Angrist [28]; Kahler [5]) did lateral lobe lesions predominate. Moore's study, indicating a 73.5% incidence in the posterior "lobe," is repeatedly cited [7-23] to indicate a predilectivity for posterior localization. It seems unlikely that the step-sectioning technique used by Moore would have resulted in an increased yield of lesions in the posterior lobe, since the over-all incidence of carcinoma he encountered was lower than that observed in our series. Actually, there is considerable evidence to indicate [3,5,24,26] that no sharp demarcations exist between the "lobes" of the prostate and what appears to be posterior lobe in one plane may appear to be lateral lobe in another. The results of several surveys [5,24,28] reveal higher incidences of lateral lobe prostatic neoplasm than those observed by Moore. Some authors [24,26] advocate abandonment of the allegedly artificial division of the prostate into lobes, substituting a zonal division into central, middle, and peripheral regions, with neoplasms predominating in the last location. At all events, it should be recognized that the majority of prostatic carcinomas probably do not arise or present posteriorly. This observation should serve to reduce the sense of security conveyed to the physician by the absence of nodulation or induration on rectal examination or the absence of carcinoma on needle biopsy of the posterior portion of the prostate.

The existence and distribution of acid phosphatase demonstrated in our histochemical preparations are entirely comparable to the experience of others.[33-36] Downey, Hickey, and Sharp [33] and Mathes and Norman [34] also observed disorganization of acinar pattern, in contradistinction to orderly reactivity of non-neoplastic prostatic tissue. The latter investigators felt, as do we, that the neoplastic acini generally manifested activity similar to that of normal acini, but with seemingly greater intensity because of the concentrations of neoplastic elements. Woodard and Dean [35] found biochemical evidence of lower acid phosphatase activity in glands affected by carcinoma than in normal prostate glands. Reiner, Rutenburg, and Seligman [35] investigated acid phosphatase in many neoplasms and observed that a wide range of epithelial neoplasms manifested strong activity, which, however, did not vary with the degree of differentiation of the neoplasm. Braunstein, Freiman, and Gall [37] demonstrated the presence of this enzyme in neoplastic histiocytes in both normal lymph nodes and in certain forms of malignant lymphoma.

Studies applying multiple enzyme techniques to the prostate have not been previously reported, but activities of different enzymes in the glands of humans and animals have been investigated separately.[38-42] It has been recognized that prostatic acini manifest strong nonspecific esterase activity,[40,41] a trait they share with most epithelial tissues. Past experience [34,41] has also indicated that neoplastic cells may be expected to manifest activity similar to that of their normal counterparts. This proved to be the case in prostatic neoplasms. Furthermore, the ubiquitous presence of acid phosphatase and nonspecific esterase in human tissues would indicate that little aid might be anticipated from histochemical techniques in demonstrating prostatic origin of a neoplasm whose primary source is unknown.

Summary and Conclusions

Two hundred twenty (220) prostate glands were secured at necropsy from men over 50 years of age not suspected clinically of having carcinoma of the prostate.

Each was studied by means of a single transverse large tissue section. Twenty-

seven were examined histochemically for enzymatic activity; six contained carcinomas.

Of the 220 specimens, 71 (32.2%) contained one or more foci of carcinoma; in 26 of these more than one focus was encountered; in 24 more than one lobe was involved; in 5 the entire gland was involved.

In 59 of the 66 cases (89.6%) in which the whole gland was not involved there was lateral lobe involvement; in 22 (33.3%) there was posterior lobe involvement.

The incidence is similar to that described in series in which more intensive techniques were utilized; the predominant localization in the posterior lobe in this series is at variance with most other reports.

Histochemical studies revealed marked acid phosphatase and nonspecific esterase activity of both neoplastic and non-neoplastic acini but lack of significant alkaline phosphatase or 5-nucleotidase activity; the striking disorganization of architecture in carcinoma is emphasized by enzyme studies.

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The Pulmonary Response to Certain Chronic Irritants

An Experimental Study on Rats and Guinea Pigs

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In a previous publication [1] it was pointed out that, when irritated by quartz dust, alveolar cells proliferate to form coherent cell masses that may fill or nearly fill the air spaces adjacent to small vessels. These proliferated alveolar cells may so closely resemble lymphocytes that it may become very difficult to differentiate between the two types of cells in routinely stained paraffin sections on the basis of cell morphology alone. In older stock rats and guinea pigs of this laboratory the occurrence of similar perivascular collaring by small round cells resembling lymphocytes is a common finding. Consequently, a study of these cell collections seemed indicated, particularly their relation to spontaneous disease and to the intrapulmonary deposition of various substances. Other useful data anticipated from such a study included the relationship of these cell collections to surrounding structures and the relation of the individual cells to mineral-dust deposits, as well as to the reticulin framework within and around the cell collections.

Materials and Methods

The lung sections of animals used for this study were taken from different experiments. Most of the experiments had been concerned with the response of lung tissue to the intratracheal injection of various dusts in aqueous suspension and several with the response to the inhalation of various dusts. Miscellaneous experiments included the intratracheal injection of avirulent tubercle bacilli, of cholesterol alone or adsorbed to kaolin, of corn oil, and of proteins such as serum (human) or egg albumen adsorbed to clay or asbestos dust. The dusts used included feldspar, limestone, kaolin, colloidal silica, asbestos (chrysotile), vitreous aluminum silicate, and various calcium silicates. Because the high incidence of spontaneous disease in postmature animals tended to bias the results, such old animals were generally excluded from this study.

In order to obtain additional information on the role of spontaneous disease in the formation of perivascular cell collections, the lung sections of 19 rats used for controls in long-term feeding experiments were studied. As far as could be determined from the weight curve and the gross and microscopic examination of the various organs, these animals had been in good health. A smaller number of stock guinea pigs (six were 14 months old, and three were 9 months old) were killed. As controls of a different sort, disease-free lungs of four rats 61 days old and disease-free lungs of four guinea pigs from 339 to 408 days old were obtained.* These animals had been raised under germ-free conditions at the Lobund Institute, University of Notre Dame, under the directorship of Professor Reyniers. The lungs were inflated with Bouin's fixative and paraffin sections prepared.

Altogether, the lung sections of 131 animals were studied, generally inclusive of every lobe. These included 95 rats and 36 guinea pigs. All sections had been stained routinely with hematoxylin and eosin, and in most instances replicate sections were also available stained with the Van Gieson method and for reticulin by the Gordon and Sweet method.

The interrelationship of the perivascular cells and the reticulin framework was studied by first marking particular vessels with their associated cell masses and locating their relative positions by means of a Field-finder.† After these fields were photographed, the cover slip was removed, and the section was subjected to the Gordon and Sweet method of silver impregnation. The same

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† Obtainable from W. & L. E. Gurley, Engineering Instruments, Troy, N. Y.

fields were then rephotographed. In some instances it was also of interest to determine the distribution of mineral dust within a perivascular cell mass. This was done according to the method previously published[3] by rephotographing the identical fields for the third time, after the slide had been incinerated and acid-washed. In all, 174 microscopic fields were photographed and 367 negatives studied.

The incidence of perivascular cell collections was determined by counting 100 vessels except in cases of nonuniform distribution. A total of 209 counts of 100 vessels each was made on the lung sections of the 131 animals.

Results

The incidence of perivascular cell collections varied greatly from one experimental group to the next and also within the group. However, some dusts were associated with a higher incidence of these cell collections than were others. A high incidence of perivascular cell accumulations was often noted in lungs altered by spontaneous disease, provided that this disease was of a chronic nature. In contrast, when severe acute inflammation was present in the lung, the incidence of perivascular cell masses was generally sharply reduced. Disease-free lungs from germ-free rats and guinea pigs contained no perivascular cell collections.

The perivascular cell collections varied greatly in size and shape. At one extreme, the cell collections were ring-like, only two or three cells thick; or, at the other extreme, they took the shape of a solid mass resembling a lymph follicle, and large enough to be visible to the unaided eye in the stained section. Generally all intermediate sizes were found. The smaller ones tended to have irregular, ragged outlines, whereas the larger tended to have smooth, sharply delineated, nearly circular outlines.

Careful study of the reticulin preparations and the photomicrographs indicated that the cell collections occupied alveolar spaces. These determinations were based on previously published criteria.[3] The most important of these criteria is the fact that the peripheral limit of a perivascular space is formed by the reticulum of the perivascular alveoli. Because perivascular cell collec-

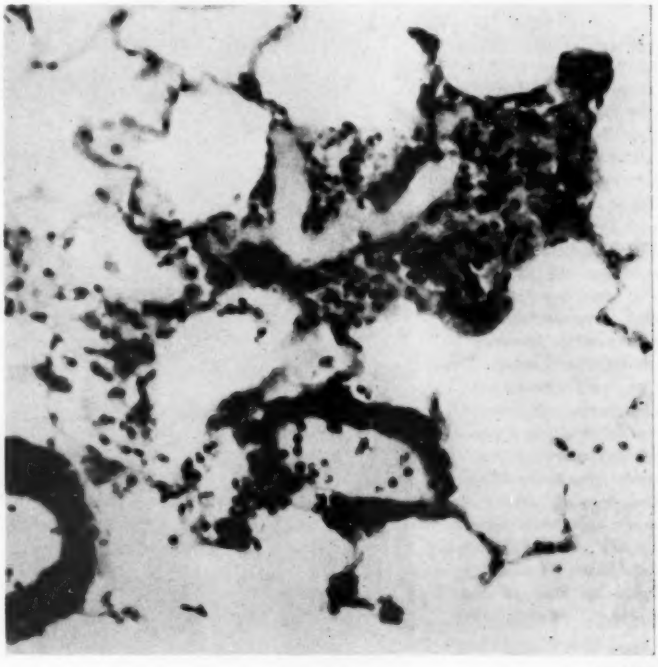

Fig. 1.—Three pulmonary vessels surrounded by multiple layers of cells. The two vessels in the lower half of the field are surrounded by small, lymphocyte-like cells, whereas the cells associated with the W-shaped vessels in the upper portion of the field are larger and have appreciable cytoplasm. There is direct continuity of these cells with similar cells of adjoining thickened alveolar walls. Section from Rat R3365, an apparently normal animal, used for diet control and killed at the age of about 14 months. Hematoxylin and eosin; reduced to 92% of mag. × 440.

tions were frequently incompletely, or not at all, delimited by reticulin fibers, it was possible to conclude that the former did not lie within the perivascular space. In the few instances in which a perivascular space was associated with a cell collection, the latter was peripheral to this space. The perivascular spaces were generally devoid of cells except in the presence of a superimposed acute inflammation. A study of the smaller cellular foci, those with ragged outlines, indicated that the irregular periphery was due to nonuniform proliferation of alveolar cells, so that the perivascular air spaces were only partially filled. In favorable situations, a solid phalanx or small lobule of cells could be seen projecting from the main cell mass into what remained of the air space. Such a projecting structure commonly had no associated reticulin fibers, either as a delimiting membrane or as supporting stroma. In some sections, the perivascular cell collections graded insensibly into thick alveolar walls, composed of clustered cells indistinguishable from those of the perivascular cell collections.

Perivascular alveoli that were partially filled with proliferating alveolar cells cammonly contained branching reticulin fibers coursing between the cells in a predominantly radial direction. In the case of the larger perivascular cell collections, the radial orientation of reticulin fibers was often obscured by the complexity of the interconnecting branches, and more particularly by thickening of lateral arcuate branches. In conformity with the sharply delineated, nearly circular character of the larger cell collections, the periphery of the latter was delimited by a well-defined reticulin fiber network, which resembled that of alveolar walls in some instances. More commonly the periphery of the large cell collections was occupied by a heavy, complex network of reticulin fibers, whereas the center would contain only sparse fibers or would be nearly devoid of such fibers, particularly where the cells appeared crowded. The large size of some of the perivascular cell masses sug-

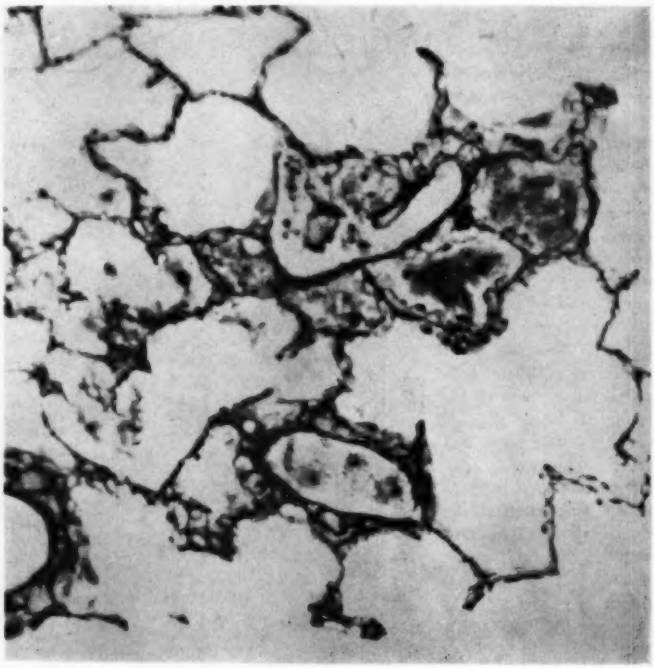

Fig. 2.—The reticulin pattern of the same field as in Figure 1. It is obvious that the cells around the W-shaped vessel are within the alveolar spaces which adjoin the vessel. These cells are associated with little or no reticulin. The smaller cells surrounding the other two vessels are associated with more reticulin. Some of the latter tends to be radially arranged. The fact that the periphery of these cell collections is not delimited by a reticulin fiber or membrane indicates that these cells are also located within the lumen of alveoli adjoining the vessels. Gordon and Sweet method; reduced to 92% of mag. × 440.

gested that not only were the alveoli immediately adjacent to the vessel involved, but also air spaces that were peripherally contiguous with the former. In some instances, as indicated by the reticulin preparations, the alveolar wall which formed the peripheral limit of the larger cell collections was in such close proximity to the wall of an adjacent alveolus that only a thin, crescentic slit remained of the latter.

The cells which made up the peri- or paravascular cell collections varied considerably in appearance. In general, three main cell types occurred: an undifferentiated cell with little or no discernible cytoplasm and resembling a lymphocyte; a well-differentiated large cell with a large amount of cytoplasm and a clearly defined cell boundary, and a partially differentiated cell with poorly defined or indiscernible cell boundary but appreciable cytoplasm. The nucleus of this last type of cell was larger, vesicular, and more ovoid than that of the undifferentiated cell. There were, of course, cells representing transitions between these main types.

The lymphocyte-like, or undifferentiated, type of cell generally had a small, darkly staining nucleus, which had a circular cross section but frequently was irregularly oval. This type of cell was most abundant in the cell collections associated with spontaneous disease but could also be found in lungs of animals containing dust of such silicates as kaolin and feldspar. The partially differentiated cell most closely resembled the cells found in the germinal center of lymph follicles except for its cytoplasm, which would contain a considerable amount of dust. It was the cell comprising most of the very early experimental silicotic nodule. It was also found in the cell collections due to spontaneous disease and in cell collections associated with the intrapulmonary deposition of the dusts of various silicates.

The well-differentiated cell was not found in the para- or perivascular cell collections due to spontaneous disease, or only infrequently in those cell foci secondary to quartz dust. On the other hand, it was the cell which, alone or as a major constituent, made up such depots as those of carbon,

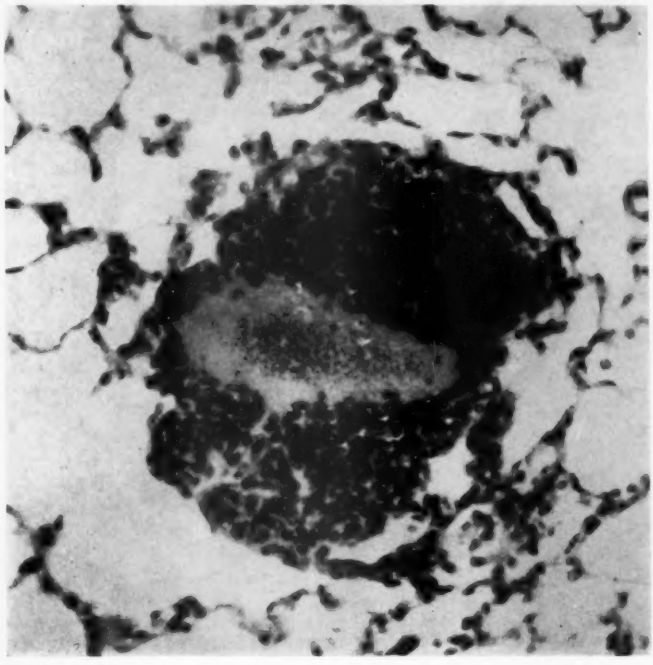

Fig. 3.—A pulmonary vessel from guinea pig G. P. 548, which had been injected intratracheally with boiled skim milk and killed two weeks later. The upper portion of the perivascular collection is composed of small, crowded, lymphocyte-like cells, and the lower part is made up of partially differentiated cells with appreciable cytoplasm. In the lower part of the field there are also transitions from the perivascular cell mass to adjoining thick alveolar walls, composed of cells identical with those in the mass. Hematoxylin and eosin; reduced to 92% of mag. × 440.

kaolin, feldspar, and others. In the case of experimental dust lesions, such as those caused by kaolin and feldspar, the cell collection contained a central cluster of large, well-differentiated, dust-bearing cells and a peripheral zone of partially differentiated cells. In some animals, there could be found interspersed among dust-containing cellular foci (inclusive of early silicotic nodules) occasional non-dust-containing cellular foci, composed only of undifferentiated or lymphocyte-like and partially differentiated cells. Such non-dust-containing foci were ascribed to spontaneous disease occurring in dusted animals.

The amount of reticulin seen in the cellular foci and, to some extent, its distribution seemed to depend upon the type or types of cells comprising the cell mass. The most abundant reticulin formation was associated with the partially differentiated cells, such as those found in very early experimental silicotic nodules and on the periphery of experimental feldspar-, as well as some kaolin-dust foci. The undifferentiated, or lymphocyte-like, cells seemed to be associated with variable reticulin production. The amount of reticulin associated with the well-differentiated cells was generally very limited.

In sections where severe edema was present, the cells of a perivascular cell collection were widely separated and reduced in number. The radial arrangement of reticulin fibers was accentuated by an apparent loss of or reduction in lateral arcuate branches. The impression was gained that with the loss of the lateral fibers the cells of the perivascular collection were readily carried into adjoining air spaces by the flow of edema fluid. Whereas the lateral walls of perivascular alveoli were frequently obscured by the complex network of reticulin fibers elaborated by the proliferated alveolar cells, in the presence of severe edema such fibers appeared less robust, and the alveolar walls consequently were more readily identified.

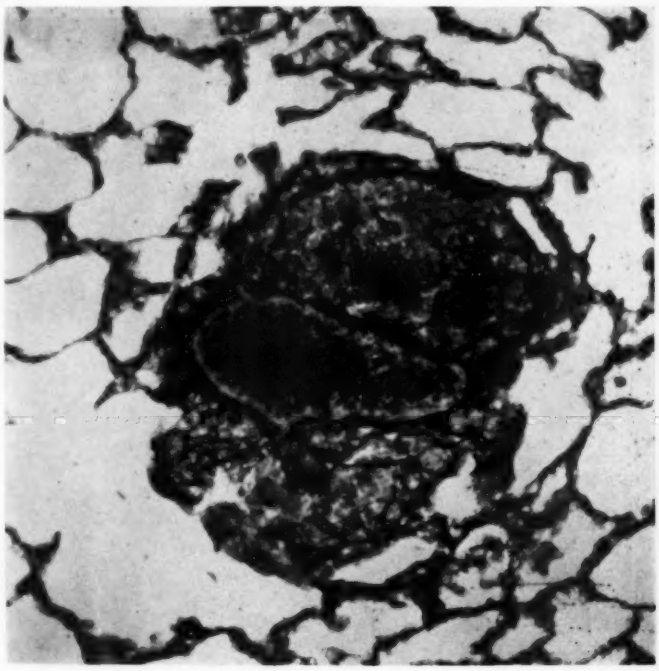

Fig. 4.—The reticulin pattern of the same field as in Figure 3. The generally radial orientation of the perivascular reticulin fibers is well shown. It is also apparent that the cells lie within air spaces which abut on the vessel. Note the small phalanx-like mass of cells without reticulin in the left lower portion of the cell mass. Reticulin fibers are scanty in the upper portion of the cell collection. The cellular proliferation here has filled the alveolar lumen completely and has pushed the alveolar wall into adjoining alveoli, so that only narrow crescentic spaces remain. Gordon and Sweet method; reduced to 92% of mag. × 440.

Comment

Generations of experimentalists have observed, and understandably have identified, the perivascular collections of small round cells in the lungs of guinea pigs and rats as lymphocytic. Thus, the mere demonstration of these cell collections is nothing new to the experimental pathologist, who believes (as did one of us [P. G.]) that such "lymphoid tissue" is so common as to be considered almost normal, particularly for the guinea pig. Nevertheless, Schottelius,[4] as early as 1878, expressed the opinion that perivascular collections of small round cells are not normal lymphoid foci but are of inflammatory origin. He based his belief upon the inconstancy with which such foci were found in rabbits and upon the frequency of their occurrence in sheep in which there was obvious disease. Almost 50 years ago, however, Miller[5] carefully described the lymphoid tissue of the lung and included periarterial, as well as perivenous, collections of small round cells as normally occurring lymphoid foci. This concept was reiterated in his relatively recent book.[6]

The observations recorded here suggest that it is advisable either to place a different interpretation on the pulmonary perivascular cell collections or to modify the present concept of the function and behavior of lymphocytes or both. Specifically, the following observations are at variance with presently accepted concepts:

1. Since the lymphocyte-like cells lie within alveolar spaces, and not within the so-called perivascular space,[3] they would appear to be somewhat anomalous as lymphocytes, because nowhere else in the animal body may a solid mass of lymphocytes be found projecting nakedly, i. e., without an epithelial surface covering, into a body cavity.

2. The cells form coherent masses which are attached to, and constitute an integral part of, the alveolar walls abutting on the vascular adventitia. On the other hand, lymphocytes are not known to be

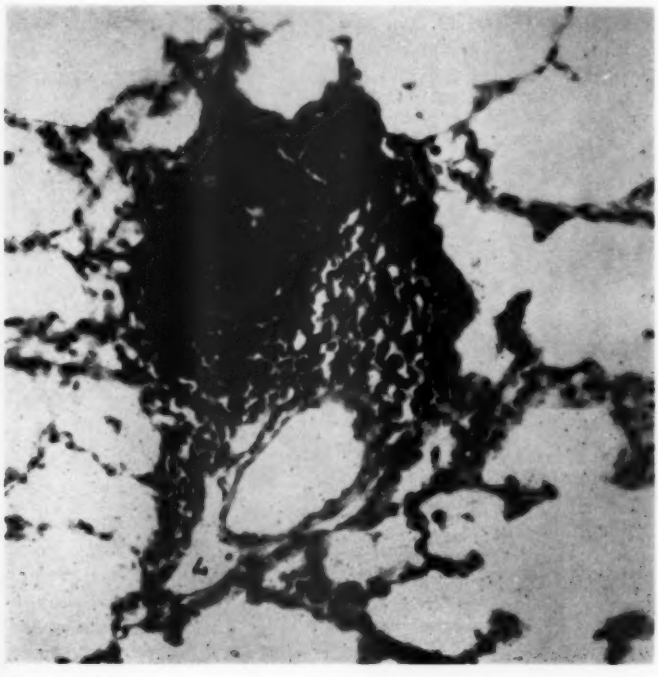

Fig. 5.—A pulmonary vessel with associated perivascular cell collection from guinea pig G. P. 272. This animal was injected intratracheally with avirulent tubercle bacilli and killed 16½ months later. No evidence of a tuberculous inflammation was found. The density of the lymphocyte-like cells in the collection is such that the nuclei appear to overlap one another. A distended, V-shaped lymphatic vessel (*L*) is present in the lower left portion of the field. Note that the cell collection is peripheral to the lymphatic vessel. Hematoxylin and eosin; reduced to 92% of mag. × 440.

coherent or to be capable of forming coherent sheets or masses.‡

3. The perivascular cell collections may merge and be continuous with thickened alveolar walls that are composed of cells identical with those around vessels. Whereas lymphocytes may conceivably infiltrate thickened alveolar walls, the ability of the cells in question to adhere to alveolar surfaces (where these cells may be prominently situated), particularly after the intratracheal injection of fixing fluid, suggests capabilities hitherto not recognized in lymphocytes.

4. Many of the cells in some of the perivascular collections contain abundant cytoplasm without discernible cell boundaries. Although so-called "young" lymphocytes may exhibit considerable cytoplasm in smears, in sections the lymphocytic cytoplasm generally appears nonexistent. Therefore, the large amount of cytoplasm found in many of the cells suggests that they are other than lymphocytes.

5. All gradations of cells, from the undifferentiated, lymphocyte-like to the large, pale, macrophage-like cell, can be identified in some perivascular cell collections. The interpretation that all of these cells are lymphocytes may be objected to.

6. The presence of dust particles within the partially differentiated cells is at variance with the generally accepted concept of lymphocytic function.[7]

7. The complex network of reticulin fibers which may surround many of the cells individually is not generally considered characteristic of lymphocytes.

8. The peripheral portions of some of the perivascular cell collections show no delimitation by reticulin fibers. If the view that these cells are lymphocytes is allowed to prevail, then the defects in the surface reticulin of the cell collections will remain unexplained.

It may perhaps be possible to reconcile the above inconsistencies and anomalies by

‡ We have been unable to document this statement; but, nevertheless, we believe it to be correct. If lymphocytes were cohesive, they would agglutinate and cause circulatory obstructions.

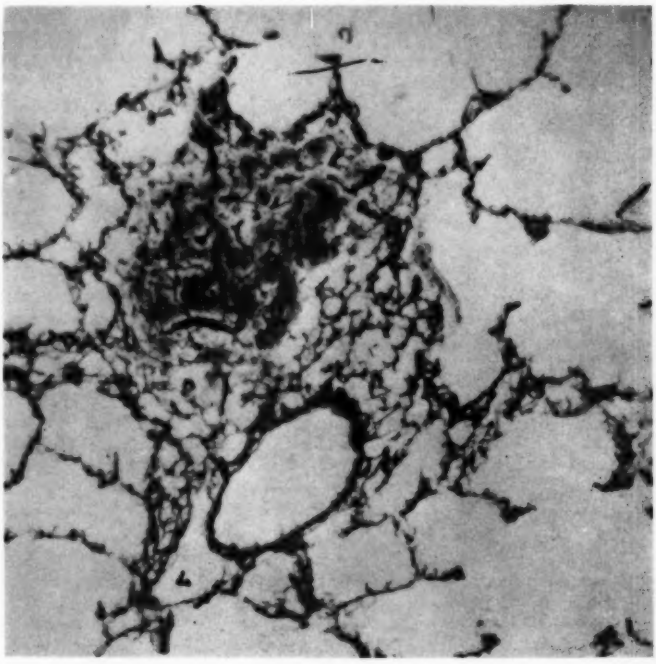

Fig. 6.—The reticulin pattern of the same field as in Figure 5, showing paucity of fibers in the region of dense cellularity (rapid proliferation). Note the lack of delimitation over the upper left convex surface of the cell mass. The reticulin fibers about the vessel show a general radial orientation. Gordon and Sweet method; reduced to 92% of mag. × 440.

a consideration of Waddell's concept [8] of the alveolar cell as one which is multipotential, and therefore capable of differentiating into lymphocytes or surface-covering cells, as well as into other cells. In terms of Waddell's concept,[8] then, perivascular cell collections may represent proliferations of alveolar cells. These perivascular proliferations of alveolar cells may vary greatly in architecture, depending upon the type and degree of differentiation. The functional activity of the cells may vary as much as the morphology. Thus, the periphery of the lymphocyte-like-cell collections may be considered to be undifferentiated alveolar cells with alveolar-cell function, whereas in the center such cells may be differentiated into lymphocytes.

The concept that alveolar cells may proliferate to form a coherent mass integrally attached to the alveolar wall was first formulated by Hulse [9] to explain the formation of small plaques of carbon deposits in the lungs.

It has been a common experience in this laboratory that animals which had been exposed to silicate dust by inhalation, or which had been injected with such dust intratracheally, usually gave very little evidence of the dust in lung sections altered by severe acute exudative inflammation. This experience also extended to animals with quartz dust in the lungs, provided that the lapse of time between dust exposure and the development of pneumonia was too short to allow for the formation of collagen. This finding has a parallel in the present study because perivascular cell collections were rarely found when severe acute pneumonic changes were present. The mechanism by which the dust foci and cell collections disappear probably involves the attenuation and actual disappearance of some of the reticulin fibers, particularly of the lateral arcuate branches, under the influence of the acute inflammatory changes (Fig. 8).

Except for the presence of demonstrable particulates and the presence of the larger macrophage-type cells, no essential difference exists between the perivascular cell collections due to dust, i. e., the *early* dust lesions, and the cell collections that occur in ap-

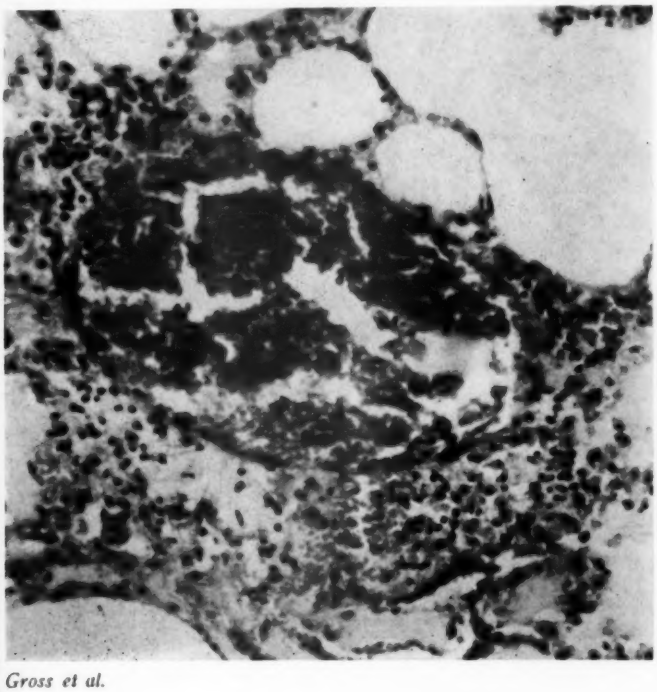

Fig. 7.—A pulmonary vein from guinea pig G. P. 270. This animal died 12½ months after an intratracheal injection of avirulent tubercle bacilli. No evidence of a tuberculous inflammation was found. The tissues and air spaces around most of the circumference of the vessel are inundated with edema fluid, which contains scattered cells. Most of these resemble lymphocytes, but some of the cells possess appreciable cytoplasm and are larger. Red blood cells are also present in the fluid. Hematoxylin and eosin; reduced to 92% of mag. × 440.

parently normal stock animals. The etiology of the latter lesions in apparently normal animals can only be speculative at present.

The formation of an intra-alveolar cohesive cell mass by the proliferation of alveolar lining cells with associated reticulin fibers leads to the obliteration of the air space, and therefore may be designated as obliterative alveolitis. The obliterative alveolitis caused by quartz dust is very similar except for one fundamental difference.[1] This difference is the subsequent development of collagen throughout the intra-alveolar cell masses containing quartz dust and the continued absence or near-absence of collagen from the cell masses caused by most other factors.

Summary and Conclusions

A high incidence of perivascular cell collections was found in rats and guinea pigs injected intratracheally with various materials. Such cell collections were also common in apparently normal stock animals but were absent from the lungs of rats and guinea pigs raised under germ-free conditions at the Lobund Institute of the University of Notre Dame.

Cell collections about pulmonary vessels were found with a high frequency in animals exposed to, or injected intratracheally with, various dusts. Some dusts were associated with a considerably higher incidence of such cell collections than others. However, although many of the cell collections in the dusted lungs contained dust, some did not.

In the presence of spontaneous chronic pulmonary disease, the incidence of perivascular cell collections was likely to be high, whereas in the presence of acute pulmonary inflammation this incidence was generally sharply reduced.

By means of reticulin stains, it has been possible to demonstrate that these cells lie, not within perivascular spaces, but within alveolar spaces. The cell collections are attached to, and form an integral part of, the alveolar walls which abut on the vascular adventitia. Evidence is adduced to support

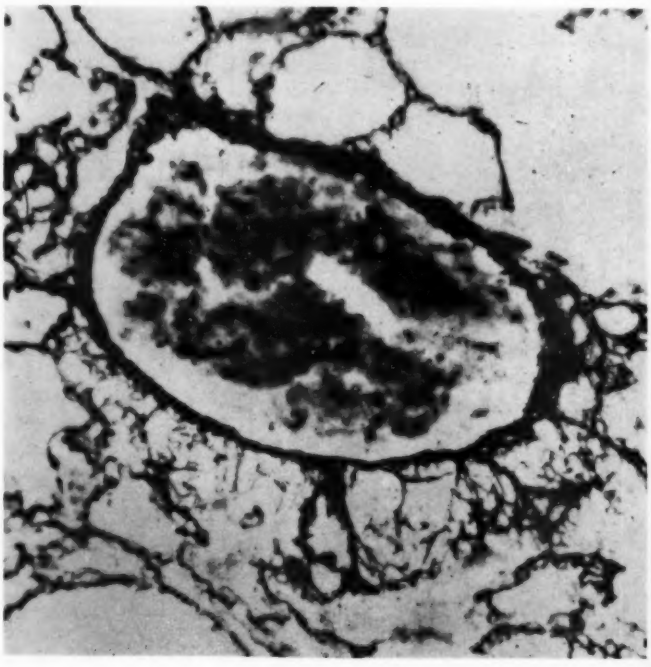

Fig. 8.—The reticulin pattern of the same field as in Figure 7, showing clearly the predominantly radial orientation of the fibers. There is apparent attenuation of all fibers, but mostly of the lateral arcuate branches. The result is that the perivascular alveolar spaces are again discernible. Reduced to 92% of mag. × 440.

the thesis that such cells are proliferated alveolar cells. In conformity with Waddell's concept that the alveolar surface cell is multipotential, the pleomorphism of perivascular cell collections may be explained on the basis of different degrees of differentiation of the proliferated alveolar cells.

Evidence has also been obtained to indicate that perivascular cell collections may disintegrate under the influence of severe acute inflammation in the immediate vicinity. This disintegration is concomitant with attenuation and partial disappearance of the reticulin framework associated with these cells.

We wish to express our gratitude to Dr. Howard T. Karsner for encouragement and valuable criticism, and to Miss Ethel Tolker, B.S., M.T. (A.S.C.P.), for her conscientious effort and meticulous care in the preparation of the sections and incinerated slides.

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Pulmonary Mucormycosis Complicating Cushing's Syndrome

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Since the comprehensive review by Baker in 1956,[1] four additional cases of pulmonary mucormycosis have been reported in detail.[5,6,9,11] Of the five instances of pulmonary mucormycosis reported by Keye and Magee,[8] three were considered slight, and only two moderate; and none was believed to be clinically significant. A majority of the recorded cases of mucormycosis have been associated with poorly controlled diabetes mellitus,[1-3,5,6] but many cases have occurred in patients with myeloproliferative disorders treated by various combinations of antimetabolites and corticosteroids.[1,2,8,9,11] This peculiar multiplicity of contributory factors makes the finding of a clinically significant pulmonary mucormycosis in a patient with Cushing's syndrome of especial interest. Mucormycosis, a world-wide disease, has been studied most intensively by Baker and others at Duke University,[1,2,6,7,10] and it is from that clinic that the only case of survival from pulmonary mucormycosis has been reported to date.[6]

Report of a Case

A 54-year-old white man, an office worker, was admitted to the Hospital of the Good Shepherd on Aug. 4, 1958, with a one-year history of polyphagia, polydipsia, and polyuria. Facial and neck puffiness developed six months prior to admission and persisted. Four months prior to admission, while under topical steroid ointment therapy for a vesicular skin eruption, he was hospitalized elsewhere for the sudden onset of exertional chest pain, dyspnea, and sweating. Myocardial infarction was confirmed electrocardiographically. After discharge he noted increased lethargy, weakness, somnolence, and wasting of extremities. He became unable to walk without assistance and eventually became bedridden. At another hospital in June, 1958, he was treated with diuretics and low-sodium diet for a 10-lb. weight gain over a one-week period. Chemical studies of the blood at that time revealed a fasting blood sugar of 500 mg. %; potassium, 2.2 mEq/L.; chlorides, 118 mEq/L., and nonprotein nitrogen, 149 mg. %. The hematocrit was 45 vol. %, and urinalysis showed a 4+ reaction for sugar and protein. He was transferred to the Hospital of the Good Shepherd six weeks prior to his death for further studies and treatment.

Physical examination on admission revealed an orthopneic man, with puffy facies, bullneck, and wasted extremities. The chest resembled, but was not fully characteristic of, a buffalo hump. The areolae were deeply pigmented. The heart was not enlarged; the pulse was both regular and irregular, with a rate of 80-85 per minute; the blood pressure was 135/80. The abdomen, although rounded, was free of striae. The liver was palpable 3-4 cm. below the right costal margin. The spleen was barely palpable. Tenderness was elicited over the dorsal lumbar processes and in the right costovertebral angle. The external genitalia were normal. The extremities were markedly wasted, and there was diminished muscular strength.

The patient was placed on bed rest, a high-protein diet, and oral potassium chloride, 6 gm. daily. Although sugar determinations fluctuated from 2+ to 4+, the blood sugar levels were kept in fair control by 35 units daily of isophane (NPH) insulin with some additional doses of regular (amorphous) insulin. The hematocrit remained constant. Albuminuria increased in severity to a level of 1.3 gm. of albumin per 24 hours. Other laboratory findings included a cholesterol of 346 mg. %, serum sodium between 144 and 149 mEq/L., and a urine Sulkowitch reaction of 3+. The protein-bound iodide was 3.0μg. %. The serology was negative. Serial white blood cell counts ranged from 4,200 to 11,500 per cubic millimeter. The prothrombin time was 100% of normal. The urinary 17-ketosteroid excretion was 14.6 mg/24 hr. The radioactive-iodine uptake was 6.2% at six hours (normal range, 7.0% to 28%), and was 6.6% at 24 hours (normal range, 10% to 50%). The resting level of plasma 17-hydroxycorticoster-

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oids was 25μg/100 ml., free, and 47.8μg/100 ml., total. After a six-hour infusion of 25 units of corticotropin, these levels rose to 40.5μg/100 ml., free, and 75.5μg/100 ml., total (normal levels, 5μg.-15μg/100 ml., free, and 10μg.-30μg/100 ml., total; resting). Radiographic studies of the skull revealed a normal sella turcica. Vertebral radiographs showed no demineralization. A diagnosis of Cushing's syndrome with hypothyroidism was made.

The patient was started on triiodothyronine, 5μg. daily on Aug. 24, and later the same dose was given twice daily. The temperature fluctuated around 99 F and reached 100 F only once. Chest films taken Aug. 13, reported as negative, showed in retrospect a small area of hazy, increased density in the left lower lung field. The patient developed a cough on Sept. 2 and coarse rhonchi on Sept. 9. Chest films on Sept. 8 showed an enlargement of the density, previously noted, and additional areas of ill-defined, hazy densities in the right lower and left middle lung fields. The patient was placed on 500,000 units of buffered procaine penicillin G given every four hours. A previous urine culture had grown out a coliform organism. Repeated blood cultures were all negative. Sputum culture yielded Proteus, Pseudomonas aeruginosa (B. pyocyaneum), and a few colonies of pneumococci. Concentration examination and cultures failed to demonstrate Mycobacterium tuberculosis. Two days prior to his death the antibiotic therapy was changed to 500,000 units of aqueous penicillin every four hours and 0.5 gm. of streptomycin twice daily. Respirations became more labored; rales and rhonchi, more prominent, and the patient died quietly on Sept. 16. He had remained afebrile throughout his hospital course. Permission was obtained for autopsy, exclusive of the head.

Gross Autopsy Findings

The autopsy was performed by me 12 hours post mortem. The general habitus was consistent with Cushing's syndrome. The right adrenal weighed 24.3 gm., and the left adrenal weighed 19.8 gm., a total of 44.1 gm. There was partial postmortem separation of the medullary zones. The cortex was uniformly thickened, pale yellow-brown, and was not greasy in appearance. No gross nodularity was present, and no accessory adrenal tissue was found along the urogenital ridge, in the renal capsules, or in the bladder trigone.

The heart weighed 420 gm. The wall of the left ventricle averaged 2.0 cm. in thickness. There was a discrete, firm, dark-yellow subendocardial scar on the anterior, lateral, and superior aspect of the left ventricle, measuring 7-8 cm. in diameter. The left circumflex coronary artery was 50%-75% occluded by an extensive atheroma. The aorta had numerous nonulcerated sessile plaques.

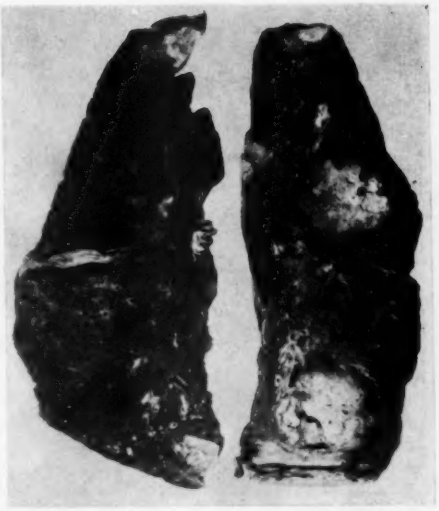

Fig. 1.—Posterior halves of lungs, showing numerous large and small necrotic cavities. Reduced to 67% of mag. × ½.

The left lung weighed 550 gm. In the left lower lobe was a cavity 5-7 cm. in diameter, containing soft, friable, caseous-like material. In other parts of the left lower lobe there were several smaller cavities, 1-2 cm. in diameter, of a similar type. Other similar areas were found in the left upper lobe. The right lung weighed 730 gm. In the apex of the right upper lobe there was a similar area, sharply demarcated by scar tissue (Fig. 1). The hilar nodes were small and intensely anthracotic.

The thyroid gland weighed 24 gm. and was grossly normal. No culture studies were attempted.

Microscopic Findings

In the adrenals the zona fasciculata was considerably thickened, and a few subcapsular cortical nodulations were present. The cortical cells were slightly pleomorphic, with rare macronuclei. No inclusions were seen. The medulla was compressed. No adenomata were found.

No interstitial cells were present in the testes, and spermatogenesis was absent.

In the heart there was an extensive subendocardial scar.

Skeletal muscle from various sites showed multiple foci of sarcoplastic vacuolation, loss of longitudinal fibrillae, and nuclear clustering. Inflammatory change was absent.

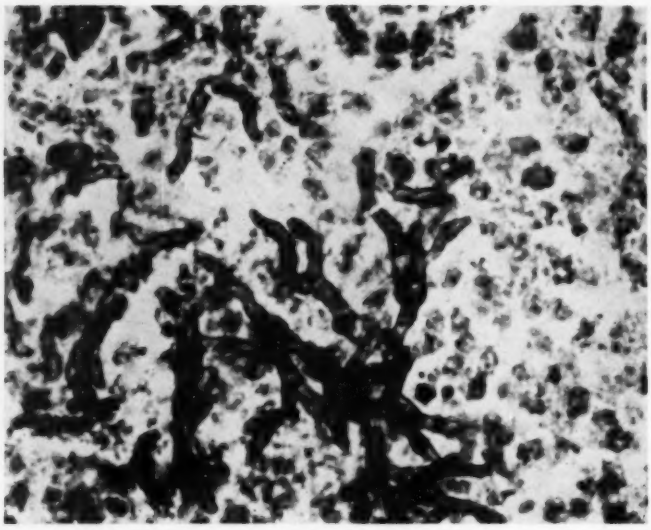

Fig. 2.—Margin of abscess, showing typical broad, branching, largely coenocytic hyphae of Mucorales. Hematoxylin and eosin; reduced to 92% of mag. × 730.

The pancreas showed no stigmata of primary-islet diabetes mellitus.

In the lungs there was an extensive inflammatory process, comprised largely of large mononuclear cells and extensive noncellular fibrinous exudate. Numerous hemorrhages and multiple foci of infarction were present. In addition to the grossly noted abscesses, many microabscesses were found. The centers of the abscesses consisted of granular, pale-staining, necrotic debris with a few broad, diffusely branching, largely nonseptate hyphae, 4μ-10μ wide and up to 200μ long in sections 6μ and 8μ thick. These hyphae took an intense blue stain in the routine hematoxylin-and-eosin preparations. On the periphery of these abscesses these hyphae were more numerous, and on the margins there were many foci of direct invasion of the walls of arteries and veins by the hyphae. Many of these vessels were thrombosed (Figs. 2 and 3). Duplicate sec-

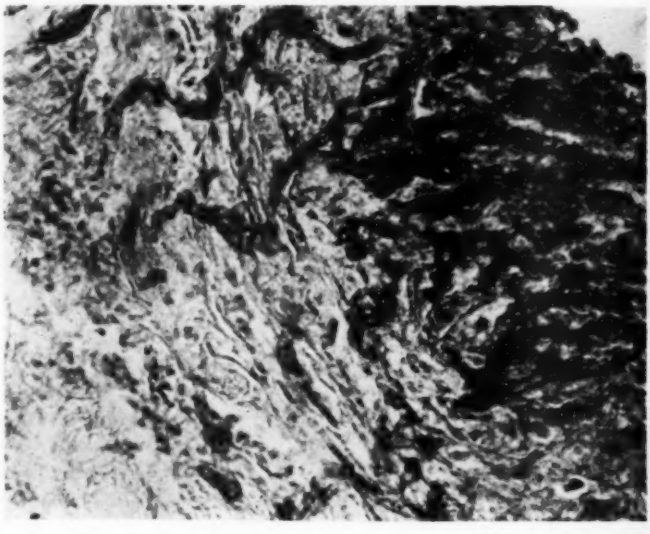

Fig. 3.—Angioinvasive activity of Mucorales. Hematoxylin and eosin; reduced to 92% of mag. × 730.

tions stained for acid-fast organisms were all negative. Periodic-acid-Schiff and Alcian blue stains did not yield any increase in contrast between hyphae and other structures. No yeast or spore forms were seen.

Two microabscesses containing identical hyphae were found in the thyroid gland, but in no other organs autopsied.

The Fungus

The hyphae in our material are identical with those described by Baker [1,2]—broad, branching, largely coenocytic filaments, measuring 4μ-15μ in width, and over 200μ in length. Hematoxylin-and-eosin preparations were adequate for demonstration of the organism (Figs. 2 and 3). Vascular invasion was prominent (Fig. 3). Although, as in Baker's cases, no culture studies were performed, it is possible to exclude aspergillosis by both the morphology of the fungus and the reaction to it. Other authors have experienced difficulty in culture of Mucorales, even in florid cases.[5] Nothing of significance can be added to Baker's discussions of pathogenesis and predisposing circumstances, or of the historical status of mucormycosis.[1,2] The general character of Mucorales, and of the experimental and cerebral forms of the disease, may be found elsewhere.[1-4,7,10] A terminal dissemination was probably developing in our patient, as evidenced by the microabscesses in the thyroid gland. In other respects, the course of the disease in our case is compatible with the clinical picture described by Baker.[1]

Summary

A case of pulmonary mucormycosis complicating Cushing's syndrome is described. The pathogenesis of the Cushing's syndrome must remain in doubt in the absence of an opportunity to examine the pituitary gland and brain. In the course of our patient's illness, inflammatory pulmonary disease developed, which progressed in spite of antibiotic therapy and fair control of the attendant diabetes mellitus. The extensive pulmonary involvement by fungus in this case is to be considered a major factor in the death of the patient. The identification of Mucorales is based on morphologic criteria.

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Addendum

Since this paper was submitted for publication, a most comprehensive review by Hutter [12] of mucormycosis has appeared.

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Congenital Aneurysm of the Left Atrium

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The present case report was deemed of interest, since only one other report of a congenital aneurysm of the atrium was encountered in a review of the literature.

Clinical History

The patient, a 69-year-old white woman, was admitted on Sept. 3, 1957, with the chief complaints of shortness of breath and severe prostration. For one week prior to admission she had had the "grippe," with generalized malaise, anorexia, and diarrhea. At the time of admission her blood pressure was 120/80; pulse rate, 110 per minute; rhythm, regular, and respiratory rate, 40 per minute. The area of cardiac dullness extended to the anterior axillary line in the fifth intercostal space. A Grade 3 apical systolic murmur was audible, and moist rales were heard over both lung bases. The clinical impression on admission was rheumatic heart disease with mitral insufficiency and congestive failure.

An x-ray film of the chest revealed evidence of cardiac enlargement in the transverse diameter and pulmonary congestion. An electrocardiogram on Sept. 4 showed sinus rhythm; rate, 100 per minute; P-R interval, 0.14 second; QRS interval, 0.8 second. Standard unipolar leads were essentially normal, with the exception of some flattening of the T-waves and isoelectric ST segments. Precordial leads, however, demonstrated slight elevation of the ST segment over the right side of the heart, but R-waves arose normally. The P-waves were not abnormal. An electrocardiogram of Sept. 9 showed essentially the same findings.

After treatment with diuretics and digitalis, she appeared to improve until Sept. 9, when she complained of nausea and was found to have distention of the abdomen and high-pitched bowel sounds. Clinically mesenteric embolism was suspected. On Sept. 10, one week after admission, the patient died.

Gross Autopsy Findings

The heart weighed 300 gm. The pericardial cavity contained almost no free fluid. Gray-white fibrous adhesions, measuring 3 cm. in greatest width, and adjacent soft gray fibrinous exudate were present over the lateral apical margin of the left ventricle. No adhesions were present over the left atrium. The foramen ovale was anatomically closed. The circumferences of the valves were as follows: tricuspid, 11 cm.; pulmonary, 7 cm.; mitral, 10 cm., and aortic, 7.5 cm. The valves of the right side of the heart were thin and delicate. There was some fusion of the commissures of the aortic cusps, the greatest extent of fusion being 2 mm. in length. The leaflets of the mitral valve had moderate fibrous thickening, and some of the chordae tendineae, particularly those of the anterior leaflet, were thickened or fused and slightly shortened. Anatomically, the mitral valve was not stenotic or insufficient.

The wall of the right ventricle had an average thickness of 3 mm., and that of the left ventricle, 15 mm. at the base. The ventral portion of the apex of the left ventricle, and the adjacent 3 cm. of interventricular septum was thinned, measuring 5 mm. in thickness. Overlying and adherent to the endocardium of the thinned portion was a red-gray thrombus, measuring 2 cm. in greatest dimension. The myocardium of the ventral portion of the apex of the left ventricle and adjacent interventricular septum was pale gray-brown with darker red-yellow discoloration of the subendocardial portion. The right coronary artery was the predominant artery and supplied blood to the obtuse margin of the heart. The coronary arteries had Grade 3 atherosclerosis with narrowing. A red thrombus completely occluded the anterior descending branch of the left coronary artery at a point 2.5 cm. from the ostium.

An oval-shaped aneurysm, measuring 4 cm. in greatest diameter and 2.5 cm. in depth, was pres-

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From the Registry of Unusual Cardiac Lesions (S. E. Gould, M.D., Director), Department of Pathology, Wayne State University College of Medicine.

Dr. Wagman was Resident in Pathology, Wayne County General Hospital, Eloise, Mich., and is now Pathologist, St. Joseph's General Hospital, Brantford, Ont., Canada. Dr. Linn is Pathologist, William Beaumont Hospital, Royal Oak, Mich., and Dr. Gould is Pathologist, Wayne County General Hospital, Eloise, Mich.

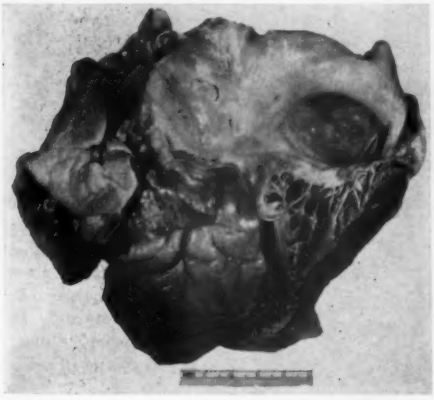

Fig. 1.—Large, oval, thin-walled aneurysm lying cephalad to the posterior leaflet of the mitral valve. Note the vertical ridges adjacent to the lateral border of the aneurysm, thickening of the mitral leaflets, and thickening and fusion of chordae tendineae of the mitral valve, especially of the anterior leaflet.

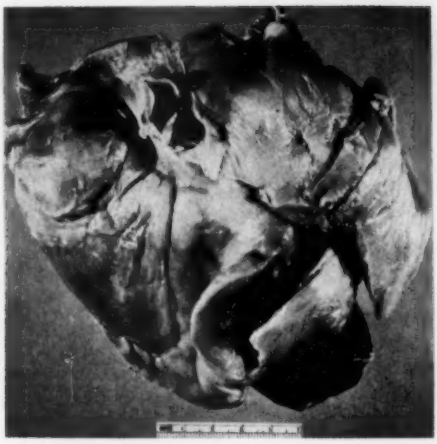

Fig. 2.—Dorsal view of aneurysm. On the epicardial surface, atrioventricular junction lies caudad and the coronary sinus cephalad to the aneurysm.

ent in the dorsolateral wall of the left atrium (Fig. 1). The aneurysm lay directly cephalad to the posterior leaflet of the mitral valve, and its wall averaged 1 mm. in thickness, was semitranslucent, and, in areas, almost paper-thin. The endocardial surface of the aneurysm was pale yellow, largely smooth, and glistening. The aneurysm extended laterally 2 cm. from the orifice of the left auricular appendage, and medially to the dorsal border of the interatrial septum. The endocardial circumferential border of the aneurysm was smooth and merged imperceptibly with the adjacent atrial endocardium, except at the cephalomedial aspect, where, for a distance of 2 cm., the junction of aneurysm and adjacent left atrium formed a sharp overhanging lip. The endocardial surface of the aneurysm was smooth except for several slightly raised, linear ridges in its deepest portion and two sharply accentuated parallel vertical ridges 5 mm. apart, measuring 3 mm. in maximum elevation and 3 cm. in length. These two ridges lay adjacent to the lateral border of the aneurysm. On the epicardial surface, the aneurysm extended caudally to the atrioventricular coronary sinus. The coronary sinus coursed in a junction and was bounded cephalically by the curved fashion along the cephalic margin of the aneurysm (Fig. 2) and appeared to have been displaced by the aneurysm from its usual position in the atrioventricular sulcus. The branches of the right coronary artery, supplying the obtuse margin, were not displaced by the aneurysm.

In addition to the aneurysm of the left atrium, the gross findings included severe atherosclerosis of the coronary arteries, with thrombosis of the anterior descending branch of the left coronary artery, recent myocardial infarction and mural thrombosis of the apex of the left ventricle, embolism of the pulmonary arteries, embolism of the superior mesenteric artery with acute infarction of the small intestine, and embolism of the left renal artery with associated renal infarction.

Microscopic Autopsy Findings

An organizing thrombus was present in the anterior descending branch of the left coronary artery. In the area of infarction in the left ventricle and septum, the myocardial fibers were replaced by actively proliferating fibroblasts, which were associated with numerous hemosiderin-laden macrophages and occasional chronic inflammatory cells. An organizing mural thrombus was adherent to the endocardium overlying the infarcted area. There were focal fatty infiltration of the myocardium of both ventricles and considerable focal interstitial fibrosis. The leaflets and chordae tendineae of the mitral valve were irregularly thickened by dense, hyalinized fibrous tissue. The leaflets of the mitral valve contained occasional small blood vessels and considerable proliferation of fibroblasts and Anitschkow myocytes, but no evidence of an active inflammatory valvular process. The epicar-

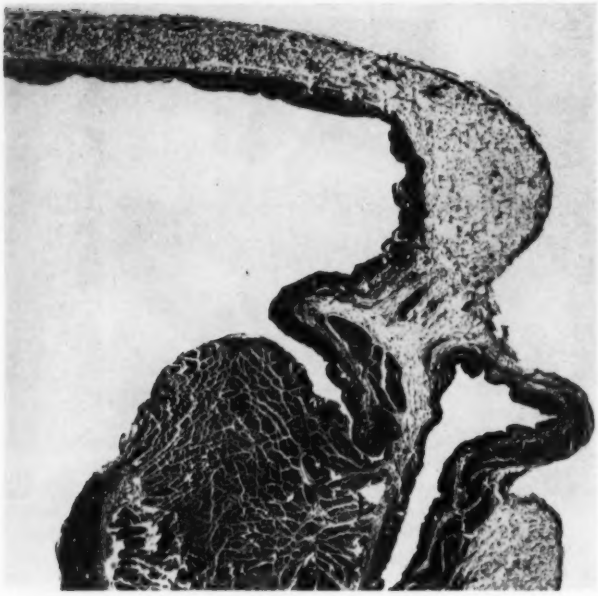

Fig. 3.—Section from cephalic border of aneurysm, showing the thinness of the myocardial layer. Note the coronary sinus at the junction of the cephalic border of aneurysm and the normal left atrium (lower right). Verhoeff-Van Gieson stain; × 15.

dium of both ventricles showed focal fibrous thickening and fibrinous exudate. In focal areas of epicardium the inflammatory response was characterized by numerous macrophages containing granular lipid material, which occasionally appeared to be fused about fat cells and often was associated with lymphocytes and plasma cells. This type of inflammatory response was present focally in the epicardium of all four chambers and in the epicardium of the left atrial aneurysm.

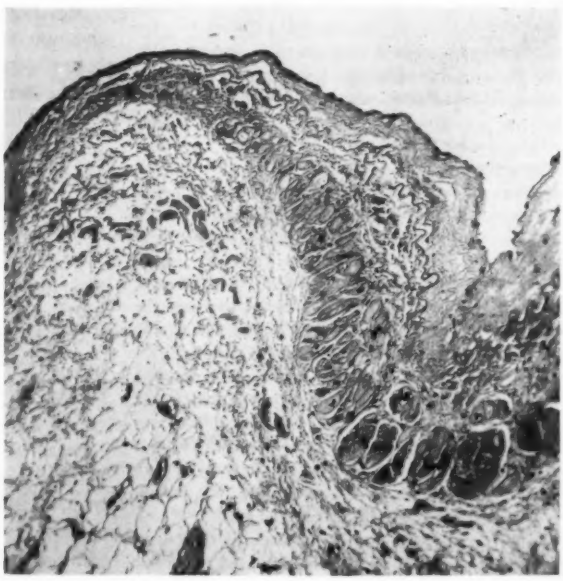

Fig. 4.—Section of aneurysm, showing absence of myocardium in wall (upper left). Hematoxylin and eosin; × 80.

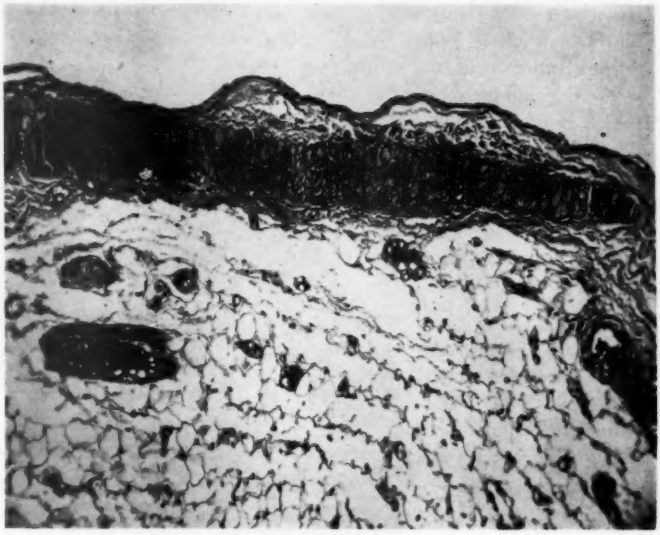

Fig. 5.—Section of aneurysm in which the myocardial layer is several fibers thick and the cytoplasm is often vacuolated. At the upper right end the myocardial layer disappears. Hematoxylin and eosin stain; reduced to 92% of mag. × 100.

In most areas the wall of the aneurysm was thin (Fig. 3), although all three layers of the heart were present. In occasional focal areas there was no intervening myocardium between the epicardial and the endocardial surface (Fig. 4). The myocardium in some areas was reduced to one or several cell layers (Fig. 5). The thinnest areas of myocardium were located adjacent to the areas of apposed endocardium and epicardium, and the thickest areas were adjacent to the junction of the aneurysm with normal left atrium. In the wall of the aneurysm adjacent to its borders, lying deep to the thin layer of myocardial fibers, a few discrete small bundles of myocardial fibers were present, surrounded by epicardial fat. The endocardial lining of the aneurysm was generally about one-fourth the thickness of the normal-appearing endocardium of the adjacent left atrium and was much thinner in some areas. In many areas of endocar-

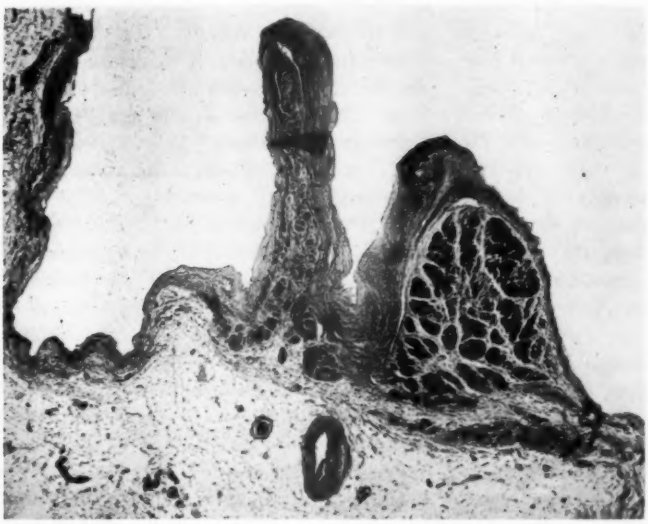

Fig. 6.—Section of aneurysm adjacent to lateral border, showing the two prominent vertical ridges composed of persistent large bundles of myocardial fibers. Masson's trichrome stain; reduced to 86% of mag. × 25.

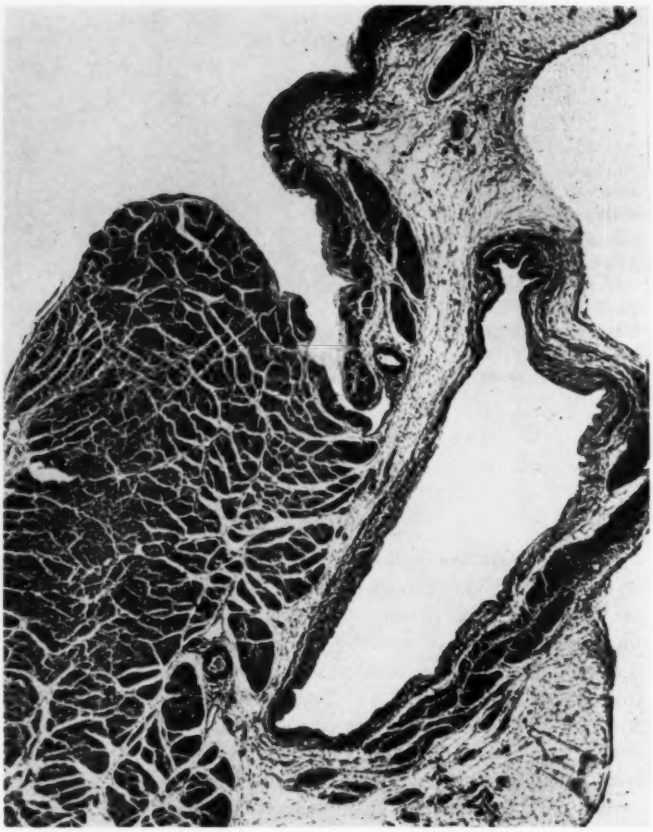

Fig. 7.—Section through cephalic border of aneurysm and coronary sinus. Note that the portion of the wall of the coronary sinus lying closest to the atrial musculature largely lacks a coat of cardiac muscle. Phosphotungstic acid hematoxylin stain; reduced to 92% of mag. × 40.

dium there was a marked decrease in elastic fibrils. The two prominent ridges adjacent to the lateral border of the aneurysm consisted of large bundles of myocardial fibers, lined by an endocardium of relatively normal thickness (Fig. 6). In the thinnest areas the myocardial fibers had lost their striations and were swollen and vacuolated. In the area of displacement of the coronary sinus, that portion of the wall of the coronary sinus lying closest to the atrial musculature largely lacked a coat of cardiac muscle (Fig. 7).

Sections of the lung revealed no evidence of chronic passive congestion.

Comment

Cardiac aneurysms, regardless of their site, can be classified as arteriosclerotic, syphilitic, mycotic, rheumatic, congenital, and traumatic in origin.

The great majority of cardiac aneurysms are the result of myocardial infarction and occur almost exclusively in the ventricles. Galindo and associates [6] encountered reports of 17 instances of congenital aneurysm of the ventricle and reported a case of their own. They accepted the explanation of this anomaly, proposed by Potts and associates,[15] that in the development of the heart the future left ventricle of the epimyocardium may accidentally fuse with the septum transversum (which gives rise to the basilar pericardium, the central leaf of the diaphragm, and the central cephalic portion of the anterior abdominal wall). As the septum transversum descends, it pulls with it the attached epimyocardium. With it, the endo-

myocardium follows the line of least resistance, and a diverticulum results.

Atrial infarctions are not uncommon (Cushing et al.[3]), but are much more frequent in the right than in the left atrium. In a study of 192 cases of atrial thrombi, Söderström[18] found 47 atrial infarcts. He found these difficult to recognize, either grossly or microscopically, because they often were associated with inconspicuous areas of complete necrosis, most probably owing to extracoronary nutrition of the thin atrial walls. In neither of these two reports[3,18] is mention made of any case of atrial aneurysm resulting from myocardial infarction. Di Ielsi and associates,[4] however, reported two cases of recent atrial infarction, one of which had resulted in the formation of an aneurysmal sac in the right atrium. In our case, the absence of scarring, even in the areas where endocardium and epicardium were apposed, and the presence of smooth ridges of myocardium at the periphery of the aneurysm would appear to preclude infarction as the cause.

Only one case of aneurysm, thought to be the result of rheumatic necrosis of the myocardium, has been described (Parkinson and associates[14]). The aneurysm was situated in the left ventricle of a 42-year-old woman who had active and healed mitral and aortic valvulitis. The wall of the aneurysm was composed of dense fibrous tissue, and a characteristic rheumatic inflammatory infiltrate was present. Interestingly enough, the aneurysm in our case was situated above the posterior leaflet of the mitral valve, in the area of the left atrium, which MacCallum found was most likely involved by rheumatic endocarditis.[10] MacCallum described the lesion in this area as a rough, corrugated, thickened patch of endocardium usually associated with thickening of the underlying wall of the left atrium and with involvement of all three cardiac layers by the process.[11] In a peremptory study, Gross[7] described the late healing stages of the "MacCallum patch" as being characterized histologically by endocardial reduplication, severe hypertrophy of the atrial myocardium, and often with myocardial fibrosis and infiltration of chronic inflammatory cells in the endocardium and myocardium. None of these features were present in our case, although the changes in the mitral valve suggest an old healed mild valvulitis of rheumatic type without apparent functional significance.

Cardiac aneurysms resulting from gummas or infective endocarditis with associated myocardial abscess are easily recognized, and these causes can be excluded in our case. Cardiac aneurysms thought to result from trauma are rare,[9] and one would expect them to be indistinguishable in their late stage from those resulting from infarction.

Endocardial pockets or pseudovalves have been reported in the left atrium,[8,16] and are always associated with severe mitral valvular disease. These are described as small folds of thickened endocardium, having a pseudovalvular appearance, with their openings facing the mitral valve. They are thought to be caused by the action of regurgitating blood, mechanically excavating an area of reduplicated endocardium, the original reduplication probably being rheumatic in origin. In these cases the wall of the excavated area is not thinned, and there is no aneurysmal outpouching. One might postulate that the force of a regurgitating stream of blood could eventually produce outpouching of a pocket and a true aneurysm. In our case, however, there was no evidence of an endocardial flap or of functional mitral insufficiency.

Thus, by exclusion, it appears most likely that the aneurysm in our case is congenital in origin. Congenital cardiac aneurysms or diverticula, exclusive of aneurysms of the interventricular membranous septum and septum ovalis, are rare.[1,2,5,12,19] Some of these[1,2] have been explained by the following hypothesis: In the stage of development of the heart corresponding to that of amphibians and fishes the cavity of the heart has the structure of a sponge with many communicating cavities. If a pinching off of

one of these cavities occurs, a cyst or diverticulum with a small opening may form. The large opening of the aneurysm in our case precludes the possibility of a pinching-off process.

In two cases other mechanisms were postulated. Drennan and van der Vijver [5] reported perforation of a diverticulum in the apex of the left ventricle of a 4-year-old child. The opening of the aneurysm was encircled by a sphincter of cardiac muscle in the form of a ridge. They noted that the center of the cortex of superficial muscle fibers situated at the left apex was the thinnest portion of the left ventricle, and constituted a weak spot where herniation might occur if the musculature at this site was poorly developed. Swyer and associates [19] reported a ventricular diverticulum in a newborn infant, occurring behind the aortic leaflet of the mitral valve. They postulated that this was caused by a defect in muscular attachment to the mitral ring at that point.

Only one instance of congenital aneurysm of the atrium (exclusive of aneurysms resulting from bulging of the redundant floor of the septum ovale) has been encountered in a review of the literature. Semans and Taussig [17] reported a congenital aneurysm of the left atrium in a 5-year-old Negro girl who had had no significant previous illness except for pertussis two months before admission. She developed palpitation, rapid pulse (noted by her mother), and shortness of breath. The heart was markedly enlarged, and a systolic murmur and thrill were detected over the precordium. Autopsy revealed an incomplete dextrocardia. An enormous saccular dilatation,

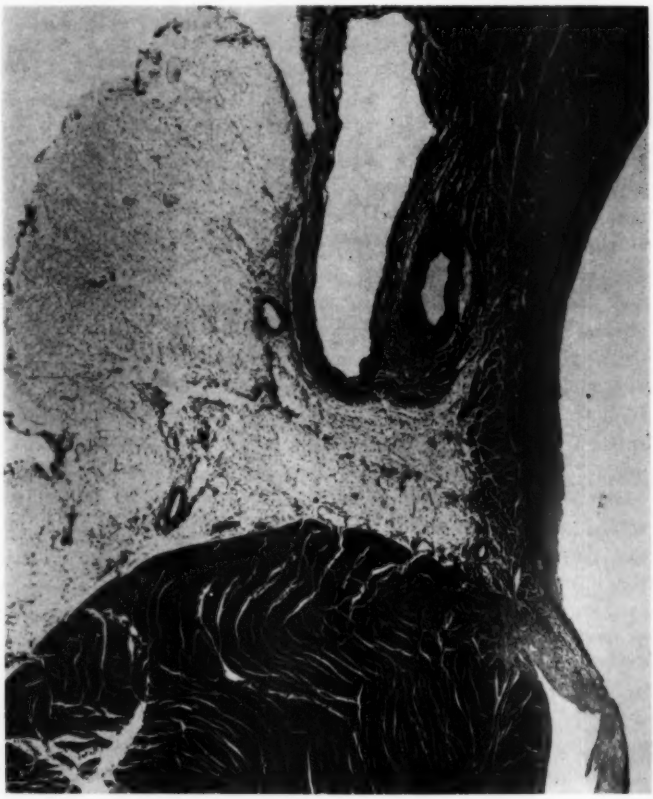

Fig. 8.—Section through left atrioventricular junction of heart of a normal 15-month-old infant. Note thinness of the left atrial musculature in the area lying between the coronary sinus and mitral ring. Hematoxylin and eosin; × 15. Courtesy of Dr. Bradley M. Patten, University of Michigan.

measuring 10 by 6 cm., involved the left atrium without extension to the appendage. The foramen ovale was completely closed; the mitral valve was slightly shortened, and the circumflex artery was extremely small. Histologically, the aneurysmal wall was markedly thin and had sparse myocardial fibers, and in some fields the fibers were completely absent. The valve and myocardium (six sections) showed no evidence of rheumatic or other infection. The authors believed their case to be one of genuine congenital aneurysmal dilatation of the left atrium, basing their belief on exclusion of other possible origins, and particularly on the presence of associated incomplete dextrocardia. The small circumflex artery was thought to be the result of embryonal dilatation of the atrium.

In our case the possibility seems likely either of a defect in muscular development or of the muscular attachment to the mitral ring. In an examination of normal hearts, it was noted that the left atrial musculature was thinnest in that area lying between the inferior border of the coronary sinus and the mitral ring (Fig. 8). Since the coronary sinus was displaced by and bordered the superior margin of the aneurysm, it is evident that the outpouching occurred in exactly that area where the left atrial musculature is normally thinnest. At that site the wall of the left atrium is composed chiefly of the left leaf of the septum primum, which sweeps along the posterior portion of the left atrioventricular ring, its lower margin being attached to the ring.[13] The coronary sinus, in its course along the posterior wall of the left atrium, is described as being partly buried in the musculature of the left atrial wall and receiving longitudinal accessions from the left leaf of the septum primum and septum secundum.[13] In our case, in the area of displacement of the coronary sinus, that portion of the wall of the coronary sinus lying closest to the atrial musculature lacked a coat of cardiac muscle. This may have been caused by a developmental muscular defect or by stripping off of muscle as the outpouching developed. Whether the aneurysm was present at birth or developed subsequently in an area that was predisposed by abnormal muscular development cannot be determined. The absence of hypertrophy and dilatation of the wall of the left atrium precludes chronic strain as a factor. On the basis of an old healed rheumatic type of mitral valvulitis, acute strain of sufficient intensity to cause outpouching of a congenitally weakened area might be postulated.

It is interesting to note that this large thin-walled aneurysm of apparent long duration did not rupture or elicit any cardiac symptoms.

The specimen and microscopic sections were referred to Dr. Maurice Lev, of the Congenital Heart Disease and Research Center, Chicago. He stated:

> The specimen in my opinion shows a mitral valve with hemodynamic changes but no evidence of inflammation. The relatively small left ventricle, the lack of distinct hypertrophy of the right ventricle, and the absence of chronic passive hyperemia of the lungs speak strongly against mitral insufficiency. Likewise, there is no evidence of inflammation in the aneurysm or surrounding atrial wall. Therefore, I favor the concept that this is a congenital aneurysm or an acquired aneurysm in a point of congenital weakness.

Summary

A large aneurysm of the wall of the left atrium, probably of congenital origin, was found in a 69-year-old woman. Only one report has been encountered of a congenital cardiac aneurysm or diverticulum of the atrium, exclusive of aneurysm of the septum ovale. The differential pathogenesis of left atrial aneurysms is discussed. It is concluded that this aneurysm was caused either by a defect in the musculature of the left atrium at that site or by a defect in attachment of the musculature to the mitral ring.

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Anorexia in Association with a Destructive Lesion of the Hypothalamus

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The experimental demonstration of hypothalamic control of eating[1,2] has focused attention on this structure as a possible site of pathologic changes in clinical situations characterized by severe aberrations in eating habits or body weight. In the case reported here, anorexia with emaciation, clinically regarded as anorexia nervosa, was the predominant clinical feature. Apart from aspirated gastric contents, which was directly responsible for the patient's death, the only significant finding at autopsy was a destructive lesion of the hypothalamus.

Report of a Case

A white woman, aged 62 at the time of her death, in July, 1952, was first admitted to the hospital in June, 1938, with severe hepatic insufficiency. Her weight then was 110 lb.; her liver was tender and markedly enlarged; her skin was icteric, and she was irrational, confused, and disoriented. A sulfobromophthalein (Bromsulphalein) test revealed 35% dye retention in 30 minutes, and her serum icteric index was elevated. Four days after admission she lapsed into coma and was not expected to live. It was learned she had been consuming considerable quantities of alcohol and was therefore thought to have Laennec's cirrhosis. Despite her critical condition, supportive and nutritional therapy was followed by gradual recovery. While improving she had delusions and hallucinations and often was incoherent. She was interviewed by a psychiatrist, who felt she had a toxic psychosis secondary to alcoholism and malnutrition. After six weeks of hospitalization she was transferred to a convalescent hospital, where she was also treated for a nutritional peripheral neuropathy. Her psychotic behavior persisted; she became extremely uncooperative and unmanageable, and, in February, 1939, was committed to a mental health institution. She responded to psychiatric therapy and was discharged. Ten months later she was readmitted because of recurrent psychotic behavior. Recovery from this episode was rapid, and she was again discharged. She remained in good physical and mental health until 1946, when a son and brother again brought her to the hospital. Laboratory findings and physical examination at this time were normal. She was adjudged a "chronic alcoholic, not insane" and care in a nursing home was recommended, which she refused. During the ensuing three years she lived alone and was seen in an outpatient department for several unrelated minor ailments. In 1949 she and her friends became aware of her failure to eat, with associated progressive weight loss. She then weighed 84 lb. Nausea, vomiting, and abdominal discomfort led to a cholecystectomy in 1950. At operation the gallbladder was found to contain several small gallstones and the liver was noted to be normal. Her weight was 80 lb. She was rehospitalized in December, 1951, with complaints of nausea, vomiting, abdominal pain, headache, and anorexia. At this time she was emaciated, weighing only 67 lb. Her physical examination and laboratory studies, including liver-function tests and multiple x-ray studies of the gastrointestinal tract, were normal. After endocrinologic and psychiatric examination, she was discharged with the clinical diagnosis of anorexia nervosa. Her final hospital admission was in July, 1952, at which time she had similar complaints, was extremely cachectic, and refused to eat. She was started on supportive therapy, including tube feedings. Two days after admission she died of aspirated vomitus.

Prior to hospitalization in 1938 she had been in good health, with an average weight of 120 lb. She had been twice married. Her first marriage, at age 22, was an unhappy one and ended in divorce at age 30. There was one child of this marriage. Her second marriage, at age 38, ended in divorce after six years, allegedly because of difficulty with her husband's family, with which she and her husband were living. It was after the failure of her second marriage that she became an alcoholic. Her menses had been normal, with the menopause at age 46. There was no family history of mental disease or emotional disturbances.

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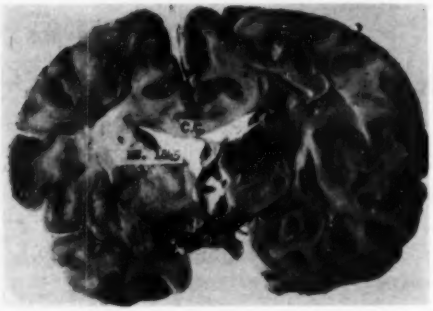


Fig. 1.—Coronal section of gross brain at level of infundibulum, asymmetrical cut. Note cystic lesion bulging into right side of third ventricle. Here, *C. C.* indicates corpus callosum; *F*, fornix; *O. C.*, optic chiasm.

Autopsy Findings

Emaciation was extreme with complete absence of subcutaneous fat. The lungs were atelectatic and microscopically revealed evidence of aspirated gastric contents. The stomach was markedly dilated, filled with greenish-brown material and showed an atrophic mucosa. The liver weighed 1,040 gm., had a smooth outer surface and a normal lobular configuration on its cut surface. Microscopically it appeared normal, with no evidence of cirrhosis or fatty alteration. The thyroid, pituitary, and parathyroids were normal on gross and microscopic examination. The adrenal glands were small and microscopically revealed atrophic changes of the cortex. Except for the brain, the remaining viscera were normal.

Brain

The brain weighed 1,320 gm. Externally it appeared normal, including the vessels of the circle of Willis. Coronal sections revealed a 15×8 mm. cystic lesion in the wall

Fig. 2.—Close-up of third ventricle at same level as Figure 1. *C* indicates cystic lesion in right side of ventricle; *I*, infundibulum; *V*, ventricle.

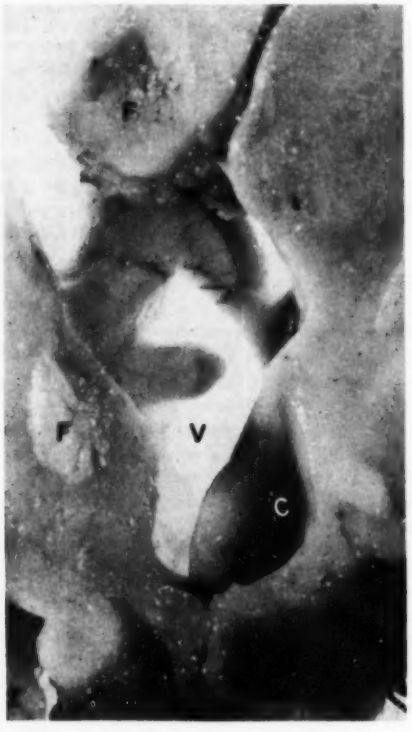
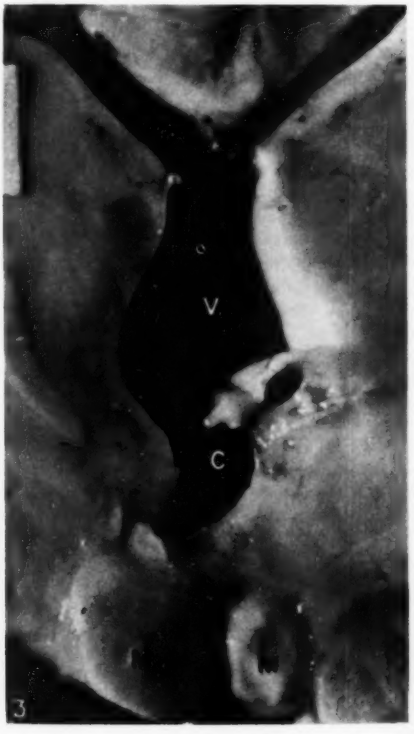


Fig. 3.—Coronal section of third ventricle at level of mammillary bodies. *C* is cystic lesion; *M*, mammillary bodies.



of the third ventricle at the level of the infundibulum (Figs. 1 and 2). The cyst involved nearly the entire right hypothalamic area and was filled with a clear, watery fluid. A thin membrane formed a common wall with the lateral margin of the third ventricle and the medial wall of the cyst (Fig. 3). It bulged into the posterior inferior portion of the third ventricle but did not obliterate it. There was a decrease in hypothalamic substance with associated prominence of the fornices. The remainder of the brain appeared normal.

Microscopic examination showed that the cystic structure was bounded medially by normal ependymal and subpendymal tissue and laterally by hypothalamic parenchyma (Figs. 4 and 5). There was minimal gliosis throughout the medial hypothalamic nuclei with a decrease in neurons. Most striking, however, was the region of the lateral hypothalamic nuclei. Here there was a paucity of neurons with minimal reactive gliosis (Fig. 6). The background stroma had a reticulated appearance, with hyperchromatic microglia and oligodendroglia nuclei and scattered large, palely stained astrocytic nuclei. No gitter cells were seen, and there was no evidence of recent or old hemorrhage. These changes were present bilaterally. Microscopic sections of the remainder of the hypothalamus were not remarkable.

Comment

Hypothalamic influence on food intake has been observed in the experimental ani-

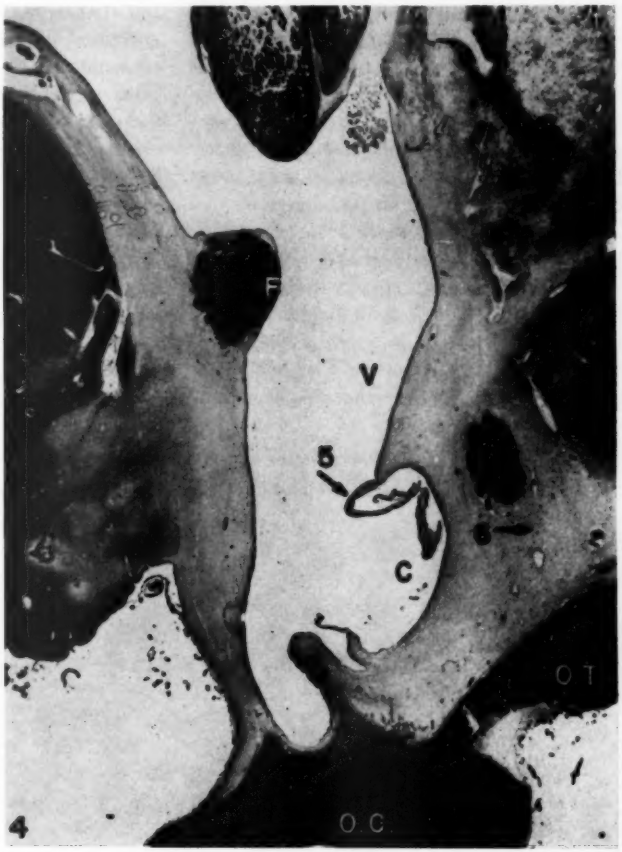


Fig. 4.—Histologic section through cystic lesion in right hypothalamus. Myelin preparation.

C indicates cystic lesion; *F*, fornix; *O. C.*, optic chiasm; *O. T.*, optic tract; *V*, third ventricle.

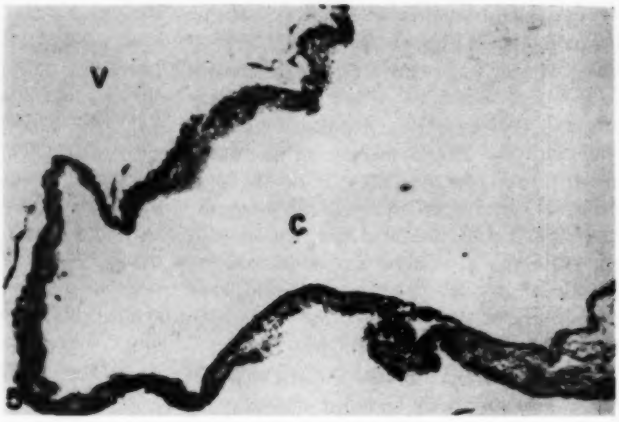


Fig. 5.—Wall of cystic lesion at site indicated in Figure 4.

mal by number of investigators.[1-9] Placement of bilateral destructive lesions in the lateral hypothalamus in rats and cats is followed by complete absence of eating,[2,9] whereas bilateral destructive lesions of the ventromedial hypothalamic nuclei produce hyperphagia and obesity.[2] Destructive lesions placed both medially and laterally produce failure to eat, and the animals starve to death.[2] Unilateral lesions in these areas do not alter eating habits.

There is evidence to indicate that these experimental observations are applicable to man.[3,6,7,10-20] Obesity frequently is reported as a symptom of hypothalamic disorders.[7,12,17] While it is often accompanied by genital hypoplasia, as in Fröhlich's syndrome,[6,11,14,15,17] or by genital hyperplasia, as in pubertas praecox,[10,15,17,20] it has also been described as an isolated phenomenon.[13,16] Emaciation or anorexia is also reported as a sequel of hypothalamic dysfunction.[6,11,13,15,20] The latter, however, occurs much less frequently. Compilation of cases reported in the literature reveals that the ratio of reported cases of hypothalamic obesity to those of hypothalamic emaciation is 4:1.[11,13,15,19,20] Several reported cases seem to confirm the rather precise localization of the hypothalamic feeding centers demonstrated in the experimental animal. Papez and Ecker [18] report a case in which an infundibuloma arising in the floor of the hypothalamus resulted initially in an "inordinate craving for sweets," followed later by a marked loss of appetite. Dott [13] also

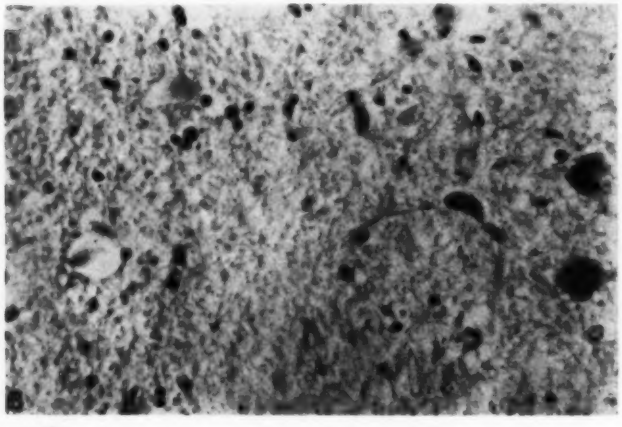


Fig. 6.—Lateral hypothalamus at site indicated in Figure 4. Note honeycombed appearance of neuropil, sparsity of neurons, and increased glial nuclei. Hematoxylin-eosin stain.

cites a case in which loss of weight was preceded by obesity. In the former case the destructive lesion arose in the floor of the hypothalamus with, first, apparent destruction of the medial nuclei, and later, as the lesion enlarged, destruction of the lateral hypothalamic nuclei. In the latter case the adiposity followed recurrence of a cystic tumor of the third ventricle, with weight loss developing after the surgical manipulation of the hypothalamus incident to removal of the recurrent tumor. The case reported here showed bilateral destruction of both medial and lateral hypothalamic areas. Its exact nature and etiology are obscure. The histologic changes suggest a process which is static or healed. We are inclined to regard it as the residue of a metabolic encephalopathy, such as Wernicke's disease or hepatic insufficiency.

Endocrinologic and psychiatric evaluation prompted a clinical diagnosis of anorexia nervosa. This is generally regarded as a functional disorder [21-28] and is an acceptable diagnosis only in the presence of a history of psychoneurosis [15,33] and in the absence of pathologic alterations which could account for the clinical manifestations.[22,25,32,34]

The demonstration of a destructive lesion of the hypothalamus would seem to negate the diagnosis of anorexia nervosa. In man, however, the presence of a hypothalmic lesion does not necessarily imply that it will produce the physiologic alterations which follow experimental lesions. Brouwer [11] and Weinberger and Grant [20] cite instances wherein hypothalmic damage was not associated with the expected clinical manifestation.

In addition to the destructive lesion in the hypothalamus, certain of the clinical manifestations cast doubt upon the diagnosis of anorexia nervosa in this case. The onset at age 59 is most unusual.[21,22,26,29,32] We have encountered but one report which includes a case of anorexia nervosa having its onset this late in life.[27] Disorder of the menses is a common finding in anorexia nervosa.[21,24-30,32] Ryle [27] claims that amenorrhea is present at some time in all cases, including those in which anorexia nervosa has its onset after the menopause. This patient had normal menses. Heterosexual maladjustment is frequently associated with anorexia nervosa.[30,32] This patient's marital history might be evidence of heterosexual maladjustment, but no statements to this effect are found in the hospital records and explanations other than this were given for the marriage failures. That she had abnormal emotional and behavioral manifestations antedating the anorexia and emaciation is quite clear. Whether or not these antedated the initial hospitalization is unknown. Behavioral and emotional disorders in man have been attributed to lesions in the hypothalamus.* If this patient had Wernicke's encephalopathy or a hepatic encephalopathy at the time of her initial hospitalization, the attendant pathologic alterations could be related to her subsequent altered behavior, either by producing it or by exaggerating preexisting abnormalities. That she suffered an acute psychosis secondary to a "toxic encephalopathy" at the time of her initial hospitalization seems certain. The temporal relationships of the clinical manifestations, plus the inability to ascertain a definite pathogenesis of the hypothalamic lesion, leave some doubt as to their exact cause-effect relations. We are inclined to regard the totality of the evidence as favoring the hypothalamic lesion as the cause of the anorexia and emaciation, and possibly as a contributing factor to her behavioral disorders as well.

Summary

A case which was characterized by anorexia, severe emaciation, and behavioral disorders, and in which a destructive lesion of the hypothalamus was the only pertinent pathologic finding is reported. The clinicopathologic implications of their association are discussed.

Department of Pathology, University of Washington (5).

* Refs. 6, 11, 13-15, 17, 20, 35-37.

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Bronchopulmonary Aspergillosis

Report of Two New Cases, Review of Literature, and Suggestion for Classification

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The Aspergilli

In 1729 Micheli adopted the name Aspergillus. He distinguished conidiophores and heads, and he noticed that the heads were rough and covered by long chains of spores; hence he gave it the name Aspergillus ("rough head"). The fungus belongs to the class Ascomycetes, order Plectascineae, family Aspergillaceae, and genus Aspergillus. Variations in names of class, order, and family are used by different workers.

The vegetative form of aspergilli consists of mycelia from which fruiting hyphae rise above the surface. The colonies of Aspergillus have characteristic colors which help in the differentiation of the various types.

The name Aspergillus has been used for molds with a conidiophore, or stalk, and a spore-bearing head. The first step in the formation of conidia is the differentiation of the foot cells. These become larger and thicker, and each usually bears a single conidiophore. The conidiophore, or stalk, is the erect, perpendicular branch from the foot cell. It enlarges toward the apex and dilates to form the vesicle. This vesicle is globose, hemispherical, elliptical, or long-clavate in various groups of the aspergilli. The lumen of the vesicle is continuous with that of the upper part of the conidiophore. The surface of the vesicle is closely covered by a layer of cells, the sterigmata. There may be one row of sterigmata, or the primary sterigmata may bear several cells, the secondary sterigmata. Each of the secondary sterigmata bears one chain of conidia. The secondary sterigmata are called phialides because they have a resemblance to a vial. Chains of spores or conidia consist of series of equal sections cut from the tip of a sterigma, and each spore is like the rest and capable of propagating the species (Fig. 8).

The genus Aspergillus includes many groups. The abundance of species in the various groups makes the identification of a specific colony extremely delicate and painstaking. The methods of identification of the different strains are generally similar to those used for other fungi and bacteria. The morphological appearance is of special significance. The size, shape, and color of the conidiophore, vesicle, sterigmata, conidia, and other components are some of the means of identification. Cultural properties and the study of colonies in the different media vary in the different groups. Biochemical activity may vary in the different strains with or without contrasting morphology. The groups comprising the genus Aspergillus are Aspergillus glaucus, A. fumigatus, A. nidulans, A. ustus, A. flaviphs, A. versicolor, A. terreus, A. candidus, A. niger, A. wentii, A. tamarii, A. flavus, and A. ocraceus. Aspergillus fumigatus is the type most frequently encountered in human bronchopulmonary aspergillosis.

Historical Review of Bronchopulmonary Aspergillosis

The subject of pulmonary aspergillosis has been gaining interest in the medical literature. The aspergilli are usually found as saprophytic organisms. It is generally understood that healthy persons are relatively immune to Aspergillus.[2] Spores of A. fumigatus have been demonstrated in the

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saliva and nasal mucus of many healthy persons picked at random. Infection with fungi (including Aspergillus) may be superimposed on other chronic diseases.[3,4] In the lung, tuberculosis, carcinoma, and bronchiectasis are the usual primary lesions. Factors of importance in the production of these infections include the administration of antibiotic drugs[4] and marrow depressants and drug addiction.

The first reference to human invasion by Aspergillus is attributed to Hughes Bennett (1842).[5,6] Subsequently, in 1856 Virchow described four cases of pulmonary Aspergillus infection in patients dying of other conditions.[5] Dieulafoy, Chantemesse, and Widal, in 1890, described the disease in pigeon crammers and gave it the name *maladie des gaveurs*.[5] The pigeon crammers of Paris took mouthfuls of grain and water and spat it into the mouth of a pigeon.[5,7,8] Rénon, in 1897, amplified these findings and also reported its occurrence in wig makers.[9] Since then sporadic cases of pulmonary aspergillosis have been published in the medical literature. A history of contact with grains and soil is demonstrated in many cases, and the occupational nature of the disease has been claimed.[10]

The diagnosis of bronchopulmonary aspergillosis is difficult, and in most of the cases it is revealed only after surgical intervention or at autopsy, as illustrated in the two cases to be reported.

Report of Cases

CASE 1.—A 64-year-old white maintenance man was admitted to this hospital on June 7, 1957, because of a weight loss of 14 lb. during the six months prior to his admission. This was accompanied by a productive cough. For three months prior to admission the sputum had been blood-stained. He had no night sweats, weakness, or anorexia.

At the age of 17, the patient had hemoptysis, and a diagnosis of pulmonary tuberculosis was made. He had another bout of hemoptysis six years prior to the present admission, and was confined to bed for six weeks. An x-ray at that time showed fibrocalcific changes in both apices. Another x-ray of the chest, in March, 1957, showed infiltration in the right apex. The sputum was negative for acid-fast bacilli. A presumptive diagnosis of pulmonary tuberculosis was made and the patient was treated for a period of four months with isoniazid, U. S. P. (Inh) and streptomycin. The lesion in the right apex showed no response.

On admission, the patient was in good general condition. He had slight clubbing of the fingers. Dullness and bronchial breathing were noted at the right apex. There was no adenopathy. The heart did not seem to be enlarged, and the liver and spleen were not felt. He had no edema. During his hospital stay he had bouts of low fever. His highest temperature was 100.0 F. Nine sputum and bronchial-washings specimens were negative for acid-fast bacilli. No tumor cells were found in the bronchial washings.

The chest film showed several calcific deposits in the left upper lobe and strands of calcification in the right upper lobe. In addition, there was a homogeneous density which obscured the right apical and subapical regions (Fig. 1).* The possibility of a stenotic malignant lesion of the bronchus with an old healed tuberculous lesion of the right upper lobe was considered. Tomography showed a circular density in the right apical and subapical region at the level of 8 cm. This lesion blended superiorly with the homogeneously thickened apical pleural cap.

Electrocardiographic examination revealed a right bundle-branch block with anticlockwise rotation of the heart on its longitudinal axis.

Bronchoscopy showed normal vocal cords. The interarytenoid area was free from ulcerations. The trachea and right main bronchus appeared normal. The middle- and lower-lobe orifices were all normal. The lower lip of the upper-lobe orifice was some-

* Reexamination of the chest films after knowing the pathologic diagnosis revealed a high light in the area of infiltration.

Fig. 1 (Case 1).—Chest film showing circular density with high light in right upper lobe.

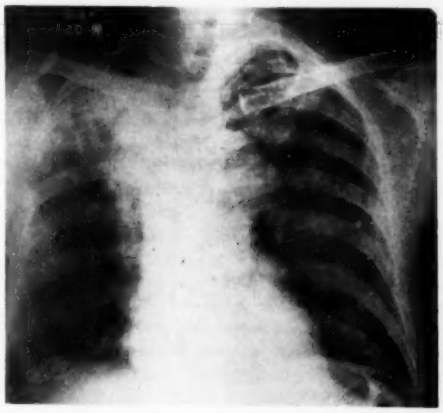


Fig. 2 (Case 1).—Surgical specimen. Cut surface of right upper lobe, showing cavity containing fungal mass.

what reddened but presented no other abnormalities. The carina pursued a somewhat oblique course, although it was sharp and freely movable. The left upper-lobe orifice, as well as the left lower-lobe orifice, appeared normal.

On July 8, 1958, right upper lobectomy was performed by Dr. A. Hurwitz. The preoperative diagnosis was "carcinoma, right upper lobe; possible tuberculoma, right upper lobe." A firm mass, measuring 5 cm. in diameter, was palpable in the posterior segment of the right upper lobe. The mass was firmly adherent to the adjacent parietal pleura. There were numerous adhesions between the right upper lobe and the parietal pleura. There were some scattered adhesions between the visceral and the parietal pleura of the middle and lower lobes. Several blebs were encountered in the apical segment of the upper lobe, as well as in the superior segment of the lower lobe. There were several small mediastinal lymph nodes present which did not appear to be involved with tumor.

The surgical specimen consisted of a right upper lobe. It measured 15×7×7 cm. and weighed 162 gm. Most of its outer surface was reddish-brown and markedly congested. The pleura generally was thin and translucent. Over the apical and posterior segments of the lobe, the pleura was thick, fatty, and fibrous. Beneath that, a palpable, firm mass occupying the apical and posterior segments was noted. It measured 6 cm. in diameter. On section (Fig. 2), the mass appeared to be formed of a large cavity lined by a thick, firm, pale-gray wall, and occupied by greenish-gray, homogeneous, semisolid material. The cavity was partially loculated, due to the presence of a few short, incomplete septa. The floor of the cavity was pale gray, and the material within was not adherent to the wall. Dilated branches of the right upper-lobe bronchus were present around the cavity, and the anterior and apical branches communicated with it. The lung tissue in the vicinity of the mass was firm and pale gray, with areas of anthracosis.

Microscopic study revealed that the cavity was partially lined by columnar and pseudostratified ciliated columnar epithelium. In some areas ulceration was noted with marked acute suppurative inflammation. The wall of the cavity and the surrounding pulmonary tissue were involved by marked granulation tissue reaction and fibrosis, with focal and diffuse round-cell infiltration. Areas of necrosis and granulomatous inflammation were also present. Fragmented bronchial cartilage was present in the areas of fibrosis. The mass in the cavity was formed of a dense network of thin-walled, septate mycelia, which showed occasional branching. Spores were also present, and the fungal filaments resembled Aspergillus. No fructification was noted. The fungal mass was either directly in contact with the epithelial lining or separated from it by a layer of polymorphonuclear leukocytes. Acid-fast stain was negative for tubercle bacilli. No cultures were taken.

The patient was seen six months after discharge. He looked well; his chest was clear to percussion and auscultation. He had gained 20 lb. X-ray showed a small air pocket in the right upper thorax.

Table 1 shows the pertinent data in similar previously reported cases.

TABLE 1 (Type 1).—*Intracavitary Aspergilloma*

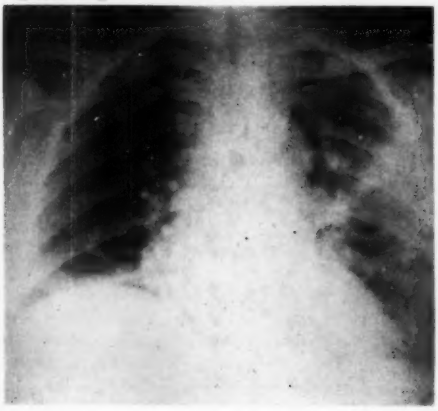
Case No.	Author & Year	Age	Sex	Main Symptoms	Chest X-Ray	Location of Lesion	Pathological Findings
1	Dévé[11] 1938	25	M	Repeated hemoptysis	Ovoid shadow with air crescent	R. L. L.	Cavity lined by bronch. epithel.; fungal mass
2	Metras & Thomas[12] 1946 *	40	M	Repeated hemoptysis	Ovoid shadow with air crescent	R. U. L.	Cavity with mycelial mass
3	Gerstl et al.[13] 1948	33	F	Repeated hemoptysis	Cavity	L. U. L.	Cavity lined by epithel.; mycelial mass
4	Yesner & Hurwitz[3] 1950	35	M		Ovoid shadow with highlight (?)	L. L. L.	Cavity lined by epithel., with Aspergillus
5	Hochberg et al.[14] 1950	48	M	Repeated hemoptysis	Infiltration	L. U. L.	Cavity lined by epithel., with Aspergillus
6	Weens & Thompson[15] 1950	42	F	Cough	Density; air crescent	R. L. L.	Cyst, lined by epithel., branching mycelia
7	Monod & Pesle[4] 1952	39	F	Repeated hemoptysis	Round cavity with air crescent	R. paramediastinal	Cavity lined by epithel., containing mycelia
8	Monod & Pesle[4] 1952	42	M	Repeated hemoptysis	Large density with air crescent	L. U. L.	Cavity lined by epithel.
9	Monod & Pesle[4] 1952	35	M	Repeated hemoptysis	Round opacity with air crescent	L. U. L.	Cavity lined by epithel., mycelial mass
10	Hinson et al.[5] 1952	58	M	Hemoptysis	Circular shadow (loose mass in cavity)	L. U. L.	Cavity lined by epithel., containing mycelia
11	Hinson et al.[5] 1952	57	M	Repeated hemoptysis	Shadow with translucent area	R. U. L.	Cyst lined by epithel., containing mycelia
12	Freidman[16] 1956	55	F	Hemoptysis	Infiltration	R. U. L.	Cavity lined by epithel., containing mycelia
13	Hughes et al.[17] 1956	50	M	Hemoptysis	Mass	L. U. L.	Cavity lined by epithel., containing mycelia
14	Levin[7] 1956	82	M	Ca stomach; dyspnea	Cavity with ovoid mass; changed position	R. U. L.	Cavity lined by epithel., containing mycelia
15	Levin[7] 1956	38	M	Repeated hemoptysis	Mass, air crescent	R. U. L.	Cavity containing mycelial mass
16	Levin[7] 1956	25	M	Repeated hemoptysis	Cavity; mass with air crescent	R. U. L.	Cavity containing mycelial mass
17	Vellios et al.[18] 1957	54	M		Mass, air crescent	L. U. L.	Cavity lined by epithel., containing mycelia
18	Hausmann[19] 1958	52	F	Double vision	Cavity with mass; changed position	L. U. L.	Cavity lined by epithel., Aspergillus mass
19	Fonshee & Norris[20] 1958	44	M	Hematuria	Cavity	R. U. L.	Cavity containing mycelial mass
20	Naji † 1958	64	M	Repeated hemoptysis	Circular density	R. U. L.	Cavity lined by epithel., mycelial mass

* Three other incomplete cases were also reported in this article.
† Case 1 of this report.

Comment

Most of the cases listed in Table 1 display clinical and pathological findings similar to those in Case 1. The leading symptom in 14 out of 20 cases was hemoptysis, and in most of the cases it was repetitive. Tubercle bacilli were not found in the sputum of any of these patients. Fungus mycelia were noted in sputa of Cases 8, 9, 10, and 13. An upper lobe was the site of infection in 17 cases. The lesion in each case was solitary. It had an x-ray appearance characterized by a well-defined oval or circular opacity, or a cavity containing a mass. On many occasions the presence of an area of translucency (air crescent) covering the top of the opacity was demonstrated. In describing these characteristic x-ray findings, Metras and Thomas [12] used the term *l'image en grelot*. Dévé [11] was the first to recognize that these findings were not pathognomonic of hydatid cyst, as was formerly believed. In only a few cases was change in position of the intracavitary mass noted on repeated x-rays. The pathological findings in the lobe involved were also strikingly similar. They were characterized by a cavity lined with bronchial epithelium and containing a loose mass of mycelia, identified as Aspergillus. In many cases the cavity communicated with one or more than one bronchus.

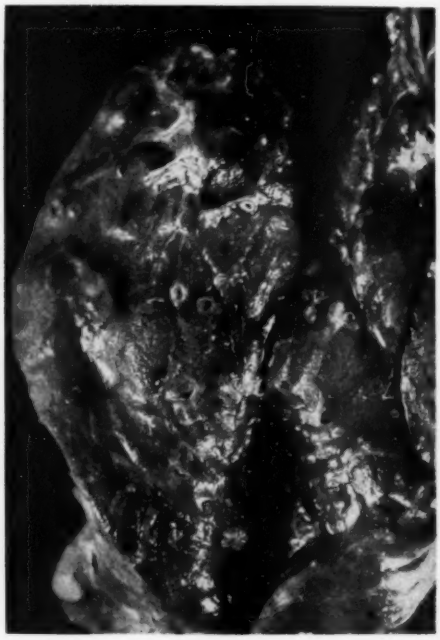
Fig. 3 (Case 2).—Chest film taken eight days prior to death, showing bronchopneumonia involving left lung.



In view of the excellent results following lobectomy in most of the cases treated surgically, excision should be the treatment of choice. Sixteen of the twenty cases in Table 1 were treated successfully by lobectomy or segmental resection. In Cases 1 and 7, death followed surgical intervention. The difficulties in arriving at a definite diagnosis solely on clinical grounds are a further indication for surgical approach.

Case 2.—A 68-year-old white woman was admitted to this hospital on March 3, 1958, with sore throat, fever, and a low white blood cell count. Early in February, 1958, she had developed upper respiratory infection with coryza and rhinitis. She was treated with Tridal (analgesic, a preparation of piperizolate methybromide and piperizolate HCl). Shortly thereafter she started to have a spiking fever temperature (103-104 F). On Feb. 6, her physician began treating her with penicillin. At that time the white blood cell count was 2,000, with 4% polymorphonuclear leukocytes. A hematologist performed a bone-marrow examination on Feb. 24 and made a diagnosis of agranulocytosis. The white cell count dropped to 900, with 1% polymorphonuclear leukocytes. A week later a bone-marrow examination showed a greater white

Fig. 4 (Case 2).—Bronchopneumonic nodules, left lung.



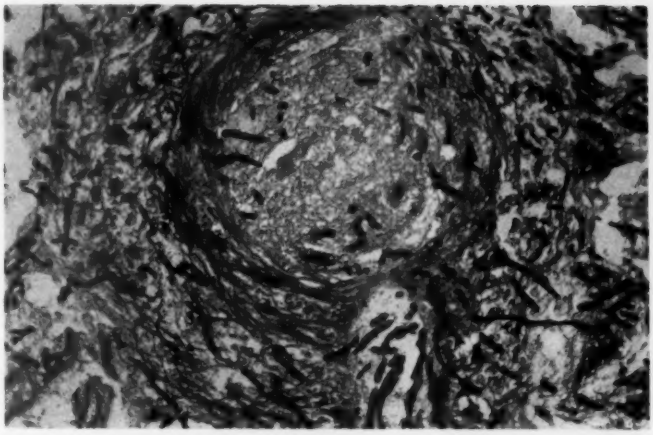


Fig. 5 (Case 2).—Photomicrograph. Aspergillus mycelia in pneumonic nodules. Mycelia also seen in thrombosed vessel.

blood cell depression. The blood count was 800 with no polymorphonuclear leukocytes. Throughout the period from Feb. 6 to Feb. 24 she was on steroid therapy.

Her past history revealed a similar episode one year prior to admission, after taking acetylsalicylic acid. At that time she responded to steroid therapy with improvement.

On admission the patient felt weak and had headache, malaise, chills, and fever. She appeared ill, dull, and lethargic with a pulse of 90 a minute and a blood pressure of 150/82. Her nose and throat were covered with a yellowish-white membrane, and her mouth was foul-smelling. She had expiratory and inspiratory wheezes. Her white blood cell count was 750 per cubic millimeter; coagulation time, 9 minutes; bleeding time, 4 minutes; hemoglobin, 9.4 gm. %; platelets, 132,000; reticulocytes, 0.7%; hematocrit, 32 vol. %, and serology, negative. The bone marrow was extremely hypocellular. Occasional normoblasts were seen. Myelocytic elements were practically absent; scattered mature plasma cells were noted. A diagnosis of marrow aplasia was made. The urine contained many granular casts. Sputum was negative for acid-fast bacilli. Nose and throat culture revealed Staphylococcus aureus. Stool and blood cultures were negative; blood urea nitrogen was 25 mg. %; glucose 112 mg. %; uric acid 2 mg. %, and the electrolytes were stable. Total protein 5.9 gm. (1.4 gm. albumin and 4.5 gm. globulin); bilirubin, 0.5 mg. %.

Chest x-ray on March 5 revealed a hazy density obscuring most of the left lung field. A diagnosis of pneumonitis was made (Fig. 3).

The patient was put on streptomycin and tetracycline (Tetracyn) with massive doses of penicillin. On March 5 the temperature remained high (102.2 F). Blood was given, and the patient was placed in an oxygen tent. Hydrocortisone was started on March 6. On March 10 streptomycin, penicillin, and hydrocortisone were discontinued,

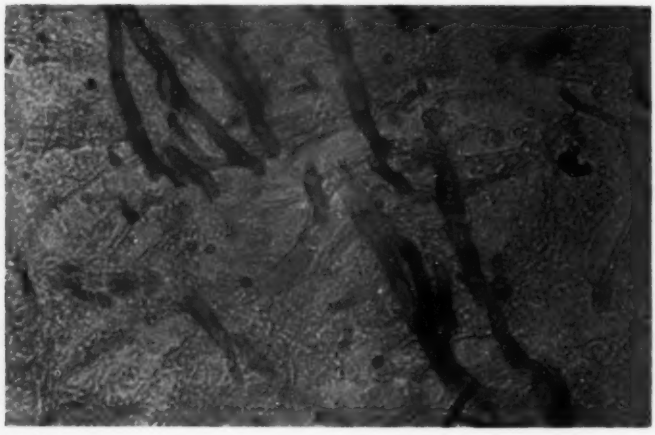


Fig. 6 (Case 2).—Photomicrograph. Septate mycelia of Aspergillus in section from pneumonic area.

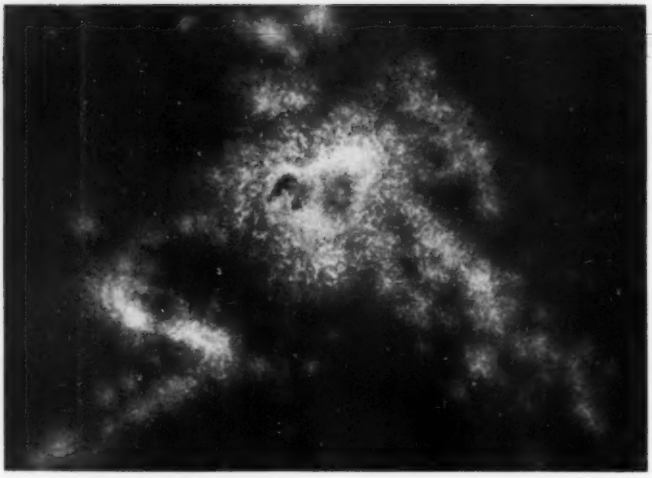


Fig. 7 (Case 2).—Culture of A. flavus in Sabouraud's agar medium.

and prednisone (Meticorten) was given with ristocetin (Spontin). She continued to have fever and died on March 13, 1958.

Autopsy revealed no pleural adhesions or effusion. The right and left lungs weighed 380 and 500 gm., respectively. Their outer surfaces were reddish-brown, and the pleura was covered by a thin fibrinous exudate. Many nodules could be palpated throughout both lungs, particularly in the left upper lobe (Fig. 4). On section, these nodules appeared as firm masses, measuring 1-2 cm. in diameter. They had a dark, reddish-brown cut surface with areas of grayish mottling. Foci of softening were noted in the center of some nodules. The margins of the nodules blended with the surrounding hyperemic lung tissue, and their gross appearance was similar to that of an infarction. Dissection of the pulmonary arteries revealed no gross evidence of thrombi.

There was a postpyloric duodenal ulcer, measuring 2.5 cm. in diameter and having a hemorrhagic base. A small amount of altered blood was present in the stomach and jejunum. Two small ulcers, each measuring 0.8 cm. in diameter, were noted in the cecum close to the ileocecal valve, and a similar ulcer was noted in the terminal ileum. The vertebrae and sternum contained pink marrow tissue.

Microscopic examination revealed that the lung nodules were formed of confluent areas of bronchopneumonic consolidation. The alveolar spaces were filled with dense, organizing fibrinous material. The central zones of the nodules showed marked tissue necrosis and distortion of the alveolar pattern. The lesions were poorly cellular, and at the periphery of each nodule there was marked intra-alveolar and interstitial hemorrhage. Within the pneumonic background a network of septate, branching filaments was noted. The mycelia were present in

Fig. 8 (Case 2).—Semidiagrammatic illustration. A. flavus fructification. Smear from culture in Figure 7.

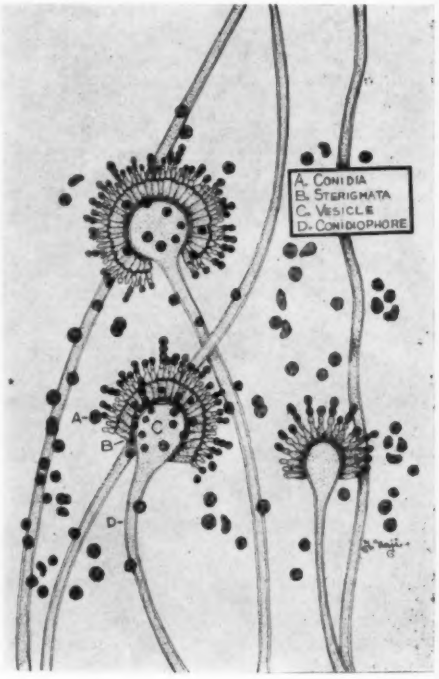


TABLE 2 (Type 2).—*Bronchopneumonic Aspergillosis*

Case No.	Author & Year	Age	Sex	Admitted for:	Chest X-Ray	Location of Lesion	Comment
1	Wahl [21] 1928	39	F	Cough and wheezing			Improvement followed iodide therapy
2	Cannon [3] 1935	15	M	Productive cough	Bilateral infiltration	Lower lobes	Improvement followed iodide therapy
3	Moolten [22] 1938	50	M	Dry cough and fever	Bronchopneumonia	Bilateral	Improvement followed iodide therapy
4	Van Ordstrand [23] 1940	36	M	Bronchitis	Bilateral infiltration	Lower and middle lobes	Improvement followed iodide therapy
5	Donaldson et al. [24] 1941	40	M	Cough and fever	Scattered infiltration	R. lower & middle lobes	Improvement followed iodide therapy
6	Coe [13] 1945	47	M	Cough and dyspnea	Emphysema and increase markings	Bilateral	
7	Coon et al. [34] 1946	65	M	Cough	History of pneumonia		Improvement followed iodide therapy
8	Cooper [14] 1946	45	M	Perforated duodenal ulcer	Bronchopneumonia	Bilateral	Patient died
9	Gerstl et al. [18] 1948	62	M	Pneumonia & hemoptysis	Infiltration	Bilateral	Infection followed agranulocytosis; patient died
10	Grekin et al. [17] 1950	22	M	Cough and fever	Pneumonitis	R. middle & lower lobes	Patient died of generalized aspergillosis
11	Ross [33] 1951	50	M	Ca of stomach		R. upper lobe	Infection followed total gastrectomy; patient died
12	Hinson et al. [6] 1952	37	M	Bronchitis and asthma	Small shadows	Bilateral	Patient died after recurrence
13	Abbott et al. [28] 1952	51	F	Bronchopneumonia	Mottled opacities	Bilateral	Patient died of aspergillosis
14	Rankin [30] 1953	45	M	Abscess of thigh	Bronchopneumonia	Bilateral	Infection followed agranulocytosis; patient died of generalized aspergillosis patient died of generalized aspergillosis
15	Welsh & McClinton [31] 1954	64	M	Hemorrhoids and fever	Bronchopneumonia	Bilateral	Patient died of generalized aspergillosis
16	Zimmerman [4] 1955	Newborn boy		Icterus		R. lung	Patient died of generalized aspergillosis
17	Najl * 1958	68	F	Sore throat and fever	Bronchopneumonia	Bilateral	Infection followed agranulocytosis; patient died

* Case 2 of this report.

varying density. They stained well with the usual stains for fungi and took faint basophilic color with hematoxylin and eosin. No sporulation was noted. Many blood vessels were occluded by thrombi, in which fungal filaments were abundant (Figs. 5 and 6).

Many culture tubes and plates were prepared, and all yielded in a rapidly growing fungus, which was identified by Dr. Chester W. Emmons † as A. flavus. The colonies were yellow-green; the conidiophores were large and spiny; the conidia were minutely spiny, and the sterigmata were in one or two ranks (Figs. 7 and 8).

Sections from the duodenal ulcer revealed a necrotic base which was covered by spores and mycelia resembling Candida albicans.

Table 2 shows the pertinent data in similar previously reported cases.

Comment

The cases in Table 2 display a different clinical and pathological picture from that in the first series. The lung lesions were in the form of bronchopneumonia with involvement of more than one lobe. Bilateral distribution was present in 11 out of 17 cases. The x-ray appearance was that of a nonspecific pneumonitis. Van Ordstrand [23] described the x-ray findings in the case he reported as having a "spider-web" or "sunburst" pattern.

Six of the patients improved after iodide therapy, while the disease was fatal to ten. It became generalized in four cases.

A. fumigatus fresenius was encountered in 10 out of 17 cases. Of the remaining seven cases, two revealed A. flavus, one A. niger, one A. sydowi, and three were of undetermined types. In seven patients the diagnosis of aspergillosis was made by finding the fungus on direct sputum examination or culture or on both. In the other 10 cases the fungus was found at autopsy in the lung lesions.

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Conclusion

It seems that two kinds of lesions can be produced by the Aspergillus organism: (1) intracavitary fungus mass or intracavitary aspergilloma (represented by the first series of cases), and (2) bronchopneumonic aspergillosis (represented by the second series of cases).

Previous authors have made attempts to classify bronchopulmonary aspergillosis by referring to "dry and wet" or "deep and superficial" types.[20] These terms do not seem adequate to describe the two types of pathological change.

As the pathogenesis of bronchopulmonary aspergillosis is not completely known, it is not always possible to determine whether the disease is primary or whether it complicates other respiratory ailments.

Summary

A review of the literature on bronchopulmonary aspergillosis is presented, and two new cases are reported.

It is emphasized that there are two types of this disease which have markedly different clinical and pathological findings. In view of these differences, an attempt has been made to classify the disease as (1) intracavitary fungus mass or intracavitary aspergilloma, and (2) bronchopneumonic aspergillosis.

I wish to express my appreciation to Dr. A. Hurwitz, Chief of Surgical Service at Maimonides Hospital, for permitting me to publish Case 1, and for his helpful advice in compiling this paper. My thanks also to Dr. Edith Neumann and Mrs. Claire L. Taschdjian, of Maimonides Hospital, and Dr. Chester W. Emmons, of the National Institute of Allergy and Infectious Diseases, for their help in the bacteriologic studies and identification of the organism in Case 2.

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Cephalothoracopagus

Report of a Case

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Numerous cases of symmetrical conjoined twins have been described. Various authors have estimated that the incidence of these monsters is in the range of 1 in every 50,000 births. Potter [1] states that only one such specimen was delivered in the course of 60,000 deliveries at the Chicago Lying-In Hospital. Grundfast and Weisenfeld [2] described the first case to appear in 50,000 births at the Brooklyn Jewish Hospital. However, only a general impression of the incidence of the various varieties of symmetrical conjoined twins has been formed because of the comparative rarity of the specimens and the variability in the morphology. Scammon [3] states that the occurrence is two to three times as common in females as in males. The current nomenclature was suggested originally by Wilder [4] in 1904, although it has been rearranged and expanded since that time. A number of cases of cephalothoracopagus have been reported, and one gains the impression that this is one of the more frequent varieties. These consist of symmetrical twins usually joined at the level of the umbilicus and above, including the thorax and head. Over 80,000 infants have been delivered at the Hartford Hospital over the period from 1945 to 1958, and this included three symmetrical conjoined twins. One of these specimens was a thoracopagus monster upon which no autopsy was performed, and another was a macerated dicephalus dipus tribrachius. Both of these were near-term in size. The following is a report of a cephalothoracopagus monster delivered at the Hartford Hospital on Oct. 16, 1958.

Received for publication Dec. 29, 1958.

From the Department of Pathology, Hartford Hospital.

Dr. William Sherpick supplied the clinical data for this report.

Report of Case

Clinical History.—The patient was a 27-year-old white woman, secundigravida, unipara, first seen on June 7, 1958. Her last menstrual period was Jan. 19, 1958, and the expected date of confinement was set at Oct. 26, 1958. She had delivered previously a normal 7 lb. 9½ oz. (3,445 gm.) female infant in January, 1955. Her past history revealed a birthmark removed from the neck at the age of 6 years. The family history was noncontributory. Physical examination showed a blood pressure of 110/80, and the heart, lungs, and breasts were normal. The size of the uterus was consistent with a 20-week gestation. Laboratory findings included an hematocrit reading of 37%, negative serologic reactions, and normal chest x-ray. The blood type was A, Rh positive. The prenatal course was uneventful until Sept. 22, when she was observed to have developed sudden abdominal enlargement with evidence of polyhydramnios. One fetal heart was heard. A lateral x-ray of the abdomen showed a single vertex and thorax, and lower extremities in the pelvis. Technique was poor, and the posterior film was clouded by the polyhydramnios. Response to diuretics, amisometradine (Rolicton) and chlorothiazide (Diuril), was poor. Pelvic mensuration revealed an adequate gynecoid pelvis.

The patient was admitted on Oct. 16, in active labor, and progressed rapidly to full dilatation, when the membranes ruptured, with release of several gallons of clear fluid. Fetal heart sounds were normal and single. After light premedication, a pudendal block was given. The patient pushed well, and a footling-breech presented. One foot pointed to the left and the other to the right. After rotation of one, it was observed that they were both left feet. Two right feet presented in the upper vagina, and, after traction and pairing, the pairs pointed to the midline, facing each other. Vaginal delivery was found impossible when traction on one pair resulted in the descent of the other. Replacement could not be accomplished. With a diagnosis of locked twins or congenital anomaly, low cervical Cesarean section was car-

Fig. 1.—Cephalothoracopagus, anterior aspect.

Fig. 2.—Cephalothoracopagus, posterior aspect.

ried out, with the delivery of an 8-lb. (3,629 gm.) cephalothoracopagus monster. Although a normal fetal heart had been heard several minutes prior to delivery, there was no evidence of life at birth, and the specimen was considered a stillbirth.

Anatomical Study.—The specimen represented a 3,200 gm. cephalothoracopagus twin monster 26 cm. crown-rump length and 51 cm. crown-heel length. The point of fusion was at the level of the umbilicus, and there was a single cord attached at this point. The thoraxes were fused in such a way as to present two opposing chests, each containing a pair of nipples, and two opposing backs. The chests and backs were at right angles to each other. A single face was present in the same plane as one of the chests, but the head was partially duplicated posteriorly. Two normal ears existed on the lateral aspects of the head, and directly in the midline posteriorly there was a dysmorphic double ear without a patent external canal. Complete external female genitalia were present in each body. There were no anomalies of the extremities, and they were exactly equal in length and diameter. For purposes of description, the side displaying the face will be referred to as anterior, and, facing the specimen in this position, the left body will be referred to as Body A and the right as Body B.

Two thoracic cavities, about equal in size, were present, one behind each chest. These cavities were separated by a thin membrane, which will be referred to in this description as the interthoracic membrane. This extended from one vertebral column to the other and probably consisted of two adherent layers of pleura because the esophagus was contained within it. A large diaphragm was present below these thoracic cavities, separating them from the large single abdominal cavity, below. Each thoracic cavity contained two laterally situated lungs and a central pericardial sac, containing a heart.

The anterior heart weighed 14 gm. and contained four chambers without any anomalies. The foramen ovale was anatomically patent. The ductus arteriosus was patent and of normal diameter. The posterior heart was much smaller and weighed 7 gm. Several anomalies were present within it and are diagrammatically represented in Figure 7. The right ventricle composed about two-thirds of the volume of the heart and communicated with a small left ventricle through a 4 mm. defect in the interventricular septum. A large pulmonary artery arose from the right ventricle, and a normal semilunar valve was present. The coronary arteries arose from the sinuses of this valve. The pulmonary artery then gave rise to bilateral pulmonary vessels to the lungs and continued through an abnormally large ductus arteriosus into the aortic arch. The aortic valve was

markedly atretic, and the aorta, arising from it, existed as a narrow cord, without demonstrable lumen, extending up to the junction with the ductus arteriosus. The pulmonary veins entered the left atrium in the usual fashion. The foramen ovale was anatomically patent.

The lungs were well formed and consisted of three lobes on the right and two on the left in each chest cavity without being mirror images of each other. The total weight of the anterior lungs was 24 gm., and that of the posterior pair 20 gm. Microscopically, the bronchioles and respiratory antra in both anterior and posterior lungs demonstrated moderate amounts of aspirated amniotic epithelial cells. Both demonstrated atelectasis of alveoli.

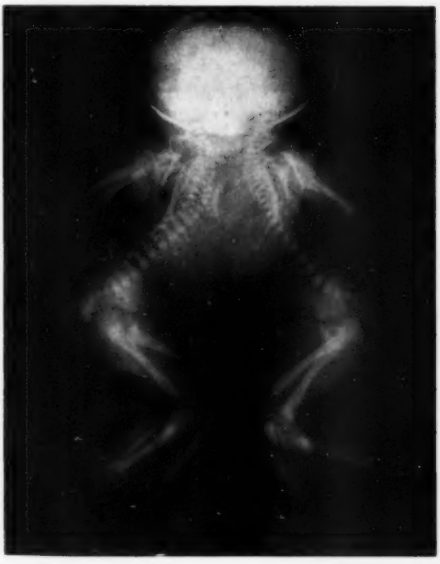
Anterior and posterior thymus glands were present but in a suprasternal position. They retained moderate right and left fetal lobulation. The anterior organ weighed 7 gm. and the posterior 6 gm. Side A of the posterior half of the diaphragm contained a large defect, through which colon and spleen protruded into the chest. There was a 1.5 cm. hiatus in the interthoracic membrane on the B side, but there was no prolapse of thoracic organs through it.

The anterior aorta arose normally and gave off innominate, left carotid, and subclavian arteries. The innominate artery was connected to the aorta of the posterior thorax by a communicating artery, which penetrated the interthoracic membrane. The right and left carotid arteries ascended into the neck and head, the internal branches eventually reaching the circle of Willis. The right subclavian artery supplied the anterior arm of Body A, whereas the left subclavian artery supplied the anterior arm of Body B. The anterior aorta continued down the vertebral column of Body B and gave off intercostal, celiac, mesenteric, renal, and iliac arteries, all supplying structures associated with Body B. Two umbilical arteries originated from the iliac arteries and entered the umbilical cord. The venous return from Body B was through a small vena cava and an unusually large azygos vein. Both of these vessels ascended into the posterior thoracic cavity to the atrium of the posterior heart. The posterior aortic arch received its blood supply from an abnormally large ductus arteriosus, as previously described. Innominate, carotid, and subclavian arteries arose from this arch. The innominate artery divided into a subclavian artery, supplying the posterior arm of Body A, and an atretic carotid artery. The two carotid arteries ascended a short distance into the posterior neck and divided into small, untraceable vessels. The communicating artery to the innominate artery in the anterior thorax arose at the apex of the posterior aortic arch. The posterior aorta continued down the vertebral column of Body A and gave off intercostal, celiac, mesenteric, renal, and iliac arteries. The visceral branches on this side were slightly larger than on the B side. Two umbilical arteries originated from the iliac arteries and entered the umbilical cord. In the proximal part of the cord two of the umbilical arteries must have fused, because microscopic section of the cord confirmed the presence of three arteries and one vein. The umbilical vein entered the substance of the liver of Body A and joined the vena cava through the ductus venosus. No umbilical vein to the liver of Body B was demonstrable. The vena cava of Body A ascended into the anterior thorax, where it joined the right atrium of the anterior heart.

The circulation to the head consisted partly of the anterior pair of carotid arteries, which entered the circle of Willis. All four subclavian arteries produced vertebral arteries, which ascended along the two vertebral columns and entered the base of the skull.

The hard palate was partially cleft, and the soft palate and uvula were entirely absent. Two Rathke-pocket recesses were present 5 mm. apart in the posterior nasopharynx, and directly posterior was a recess, which led to a duct communicating with the posterior middle ear. Two complete larynxes were present, facing in opposite directions in the anteroposterior plane. A single esophagus descended between these structures. Thyroid tissue was present anterior to each larynx. There was a very primitive tongue just above and anterior to the

Fig. 3.—Roentgenogram, anteroposterior view.



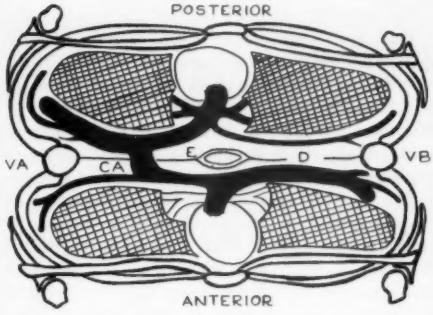


Fig. 4.—Diagram of transverse plane through chest. Note four lungs and two hearts. *CA*, communicating artery; *E*, esophagus; *D*, defect in interthoracic membrane; *VA*, vertebral column of Body A; *VB*, vertebral column of Body B.

epiglottis of the posterior larynx. The anterior tongue was normally developed.

A single esophagus descended in the midline between the two larynxes and through the center of the thoracic cavities within the interthoracic membrane. It continued through the diaphragm into a single stomach. This organ was suspended from a mesentery which extended laterally to the vertebral columns of each body and superiorly to the diaphragm. Enclosed within this mesentery were two pancreases. The stomach probably derived its blood supply from the celiac axis of both sides, but this could not be determined conclusively. The stomach continued into a single duodenum, which contained two ampullae of Vater about 1 cm. apart. The small bowel continued to a point 1 cm. proximal to a large Meckel diverticulum, where it divided into two equal branches, which extended into the separate lower portions of the two abdominal cavities. These continued into separate colons, each containing meconium. The position of the colons with respect to mesenteric rotation could not be determined because of the loose arrangement in the upper abdomen.

The liver and spleen were positioned at right angles to the orientation of the chest organs. This may be visualized by comparing Figures 4 and 5. In addition, the livers were situated in the right upper quadrant of each body. The spleens were in the left upper quadrants. The liver of Body A weighed 80 gm. and was much larger than the liver of Body B, which weighed only 50 gm. Each liver contained a gallbladder and bile ducts, which continued to the head of each pancreas, where they joined the pancreatic ducts and emptied into separate ampullae of Vater. A free flow of bile was demonstrable from each ampulla.

Adrenals, kidneys, bladders, and internal female genitalia were comparable in weight and size in the two bodies. They were below the level of fusion at the umbilicus and were symmetrically arranged, without anomalous conditions.

Two well-developed and symmetrical cerebral hemispheres were present, with no evidence of duplication. The division began in the hypothalamus just posterior to the optic chiasm. There were two pituitary stalks 3 mm. apart and duplicate pituitary glands. Two complete pontes, midbrains, and cerebellums were present and equal in size. There were bilateral sets of cranial nerves beginning with the third nerve, but the third through the seventh nerves were markedly atrophic on the medial sides and their courses could not be fol-

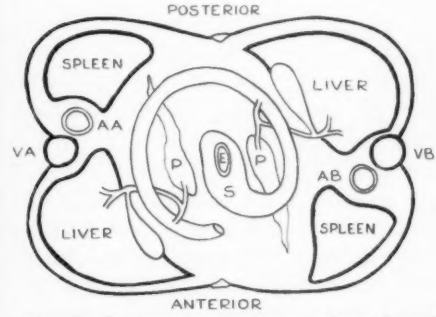


Fig. 5.—Diagram of transverse plane through upper abdomen. Schematic depiction showing single gastrointestinal tract, but duplicated liver, spleen, pancreas, and biliary ducts. *VA*, vertebral column of Body A; *VB*, vertebral column of Body B; *AA*, aorta of Body A; *AB*, aorta of Body B; *P*, pancreas; *E*, esophagus; *S*, stomach.

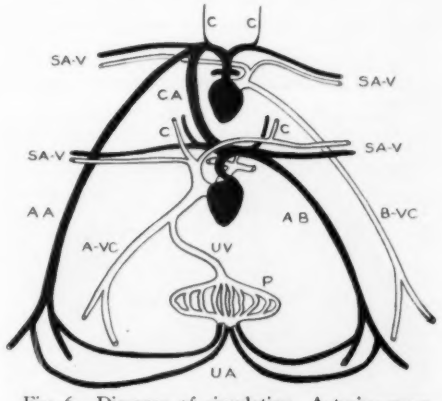


Fig. 6.—Diagram of circulation. Arteries represented in black. Posterior heart is above and anterior heart below. *C*, carotid arteries; *SA-V*, subclavian arteries and veins; *CA*, communicating artery; *AA*, aorta of body A; *AB*, aorta of Body B; *A-VC*, vena cava of Body A; *B-VC*, vena cava of Body B; *UV*, umbilical vein; *P*, placenta; *UA*, umbilical arteries.

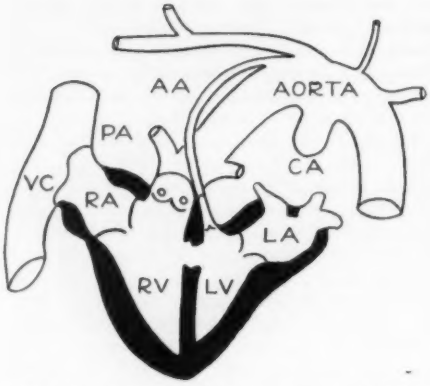
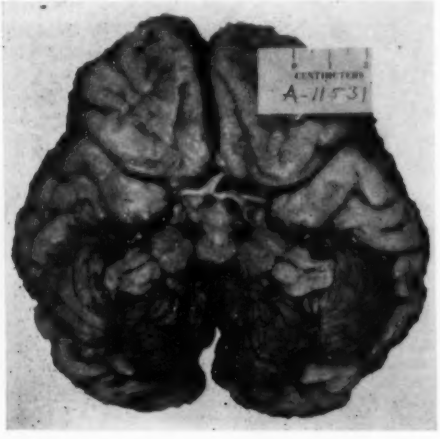


Fig. 7.—Diagram of posterior heart showing anomalies. *AA*, atretic part of aorta; *PA*, pulmonary artery; *VC*, vena cava; *RA*, right atrium; *RV*, right ventricle; *LV*, left ventricle; *LA*, left atrium; *CA*, communicating artery.

lowed. The medial vestibular and auditory branches of the eighth nerve arose bilaterally in the usual position but were somewhat smaller than the lateral components of this nerve. They entered two internal auditory meatuses in a duplicated posterior petrous ridge and progressed backward to converge in the duplicated inner-ear cavity. The exact structure of this organ was not determined except that it contained four semicircular canals and no external auditory canal. Two well-formed spinal cords descended through separate foramina magna. The circle of Willis was normal anteriorly but posteriorly was fed by two basilar arteries, each giving off a posterior cerebral artery, laterally, and a communicating artery to the other basilar artery, medially.

The two bodies contained perfectly formed skeletons except for the bizarre arrangement of the rib cage and the posteriorly duplicated bones of the skull. Each set of ribs met the opposing set of ribs from the opposite vertebral column. This resulted in the chest plane being at right angles to the plane of the back. There was marked rotation of the cervical vertebrae as the relation between the two vertebral columns changed from 180 degrees in the chest and body to about 60 degrees at the base of the skull. Duplication in the skull began in the sella turcica, and this became progressively more pronounced posteriorly. There were two separate sets of occipital bones, and portions of the temporal bones were duplicated and met posteriorly in the midline. There were two symmetrical posterior fontanelles.

Fig. 8.—Inferior aspect of brain showing single cerebrum and duplicated midbrain, pons, and cerebellum.



Comment

A large number of thoracopagus monsters have been described, but there are few reports of cephalothoracopagus monsters with detailed anatomical descriptions. Finola [5] described a case which was almost identical with this one, both externally and in the arrangement of the internal organs. This specimen also demonstrated a communicating artery between the two aortic arches, and the thoracic and abdominal viscera were duplicated, with the exception of the gastrointestinal tract, which was single down to a point 10 cm. proximal to the ileocecal valve. Gunter [6] described a cephalothoracopagus with two faces which was anencephalic and had only one well-formed chest. The thorax contained four pleural cavities but only one pericardial sac. The liver, spleen, and upper gastrointestinal tract were unduplicated. Grundfast and Weisenfeld [2] described an 1,875 gm. specimen which also had four pleural cavities, only two with lung tissue, and one pericardial sac. The upper gastrointestinal tract was unduplicated in this case also except for a partially double esophagus. There were two livers and two sets of bile ducts but only one pancreas.

It is difficult to make generalizations about the anatomy of these specimens.

Potter [1] states that the liver is united in all but the most superficially attached twins. This is not borne out by the case being described or the ones referred to above. Also, Arey [7] quotes Morrill, who stated that the viscera are often arranged as mirror images of each other and that when this is true the right-hand twin demonstrates situs inversus. This specimen showed proper rotation and right-left orientation in both halves.

One of the most interesting aspects of this specimen was the circulation. Since there was no ductus venosus on the B side, oxygenated blood would have to travel through the systemic circulation of the B body or retrograde down the ductus arteriosus before arriving at the posterior heart. This immediately invites speculation as to the effects of this undoubted hypoxic state on the anomalous development of that heart. In the face of the anomalous circulation present this fetus was able to reach term in a viable state.

It would be beyond the scope of this paper to discuss the theories of causation in the embryonic development of monsters. Numerous agents have been demonstrated to induce anomalous fetal development by experimental methods. Among these are heat, radiation, mechanical interference, chemical changes, oxygen deficiency, and hereditary influences. Viral infections, especially rubella, have been shown to alter fetal development. It was not possible to elicit a history of any significant phenomena having occurred during the first two weeks of gestation in this case. Similarly, no previous history of congenital malformations in this family was known.

Much has been written about the stage of embryonic development at which abnormal twinning occurs. Division in the inner-cell-mass stage, if complete, would result in normal monovular twins with separate amniotic sacs. If division takes place later in the embryonic disk normal monovular twins within a single amniotic sac may occur, or if division is incomplete, various types of conjoined twins. It is of interest that the first organ system to make its appearance is the primitive gut, which is derived from the yolk sac. If twinning should occur during the time that this gut is being established in its primitive form, we should expect that the gut would retain varying degrees of its singular status and that all other organ systems would show greater, if not complete, degrees of duplication. In the case being presented, and in the others referred to, the gastrointestinal tract retained a marked degree of singularity, despite extensive duplication of other systems. It seems reasonable to assume that in these cases twinning occurred late and incompletely in comparison with the earlier and complete division occurring in normal twinning.

The presence of polyhydramnios in this case supports the high incidence of this condition reported by others. Only one fetal heart was heard, and this also concurs with the findings of other authors.

Summary

A case of cephalothoracopagus in which the fetus survived a full-term gestation and weighed 3,200 gm. is reported. There was a remarkable symmetry of the specimen, both internally and externally, and a curious anomalous circulation was present. The anatomy is compared with others of this type which have been reported, and inferences are drawn concerning the embryonic development.

Hartford Hospital.

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A Rare Variant of Ameloblastoma

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Ameloblastoma is a not infrequent tumor,[15] usually occurring in the mandible or the maxilla, and rarely to be found in other bones,[2-4] neighboring the pituitary gland,[29] or in the soft tissue of the jaws.[18] According to Sharp and associates,[20] the ameloblastoma is essentially a tumor of basal-cell character. Microscopically, in the typical tumor the peripheral cuboidal or columnar cells of the neoplastic epithelial nests, strands, or sheets are palisaded, whereas the more centrally located cells are loosely arranged in a "reticulated" pattern. This gives the tumor a certain resemblance to the enamel organ of the developing tooth. For the various theories on the histogenesis of the ameloblastoma, as well as for its salient gross and microscopic features, the reader is referred to the articles by Robinson,[19] Thoma,[23,24] Sharp et al.,[20] and Small and Waldron.[21]

Boyd,[7] discussing the ameloblastoma, states: "There is no constant microscopic picture." On histologic grounds the ameloblastomas were divided and subdivided into numerous groups.* Bernier[5] recognized only four groups, namely (1) adamantine ameloblastoma, (2) monocystic type, (3) polycystic type, and (4) fibroameloblastic variant. According to the time at which the ameloblastoma arises from the enamel-forming cells during their differentiation toward the adult ameloblast, a variety of microscopic pictures is to be found. We recently examined a tumor of the maxilla, which we diagnosed as an ameloblastoma, but which did not correspond to any description found in the literature available to us. Because of the obvious rarity of this tumor, and because of its interesting biological behavior, we thought it worthy of report.

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* References 1, 6, 8, 9, 14, 18, 19, 21-28.

Report of a Case

A 13-year-old boy was referred to the maxillofacial unit of the Beilinson Hospital because of a swelling in the right side of the upper jaw of a few months' duration. A slight bulging of the buccal surface of the alveolar ridge in the region of the first premolar was found. X-ray examination revealed a radiolucent lesion of the bone in this area.

The operation was performed by S. Rotter, D.D.S., who excised a grayish-white tissue mass of soft consistency, 2×1.5×1 cm. in size. The cut surface was of uniform appearance, gritty, and of whitish color.

The various findings in the microscopic examination are illustrated in Figures 1 to 9.

Figure 1 shows a portion of the tumor, found to constitute the major part of the excised mass. The surrounding connective tissue was moderately vascularized, slightly infiltrated by round cells, and contained a few small nerves and bony spicules. The spicules consisted of young, primitive, centrally calcified bone with a relatively thick rim of osteoid.

The tumor was marked out by a distinct stratified epithelial layer (Fig. 1). The peripheral cells were cuboidal to columnar and palisaded (Fig. 2); their round to oval, chromatin-rich nuclei were centrally placed. The cytoplasm abutting on the stroma contained vacuoles (Fig. 3) and fine PAS-positive granules. In some places the basal membrane was markedly thickened, owing to accumulation of an amorphous, brightly eosinophilic material (Fig. 4), suggestive of amelogenesis.[11] The more centrally located cells were loosely arranged in a "reticulated" pattern (Fig. 3). Here and there the epithelial cells encroached upon

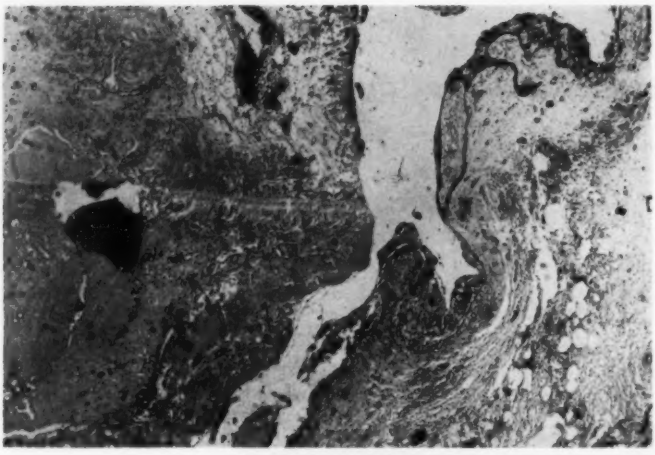

Fig. 1. — Low-power view of the tumor. Note the distinct peripheral layer of epithelial cells. To their left the central tumor mass, constituting the special feature of this tumor, is seen. (The cleft is an artifact.) Hematoxylin and eosin.

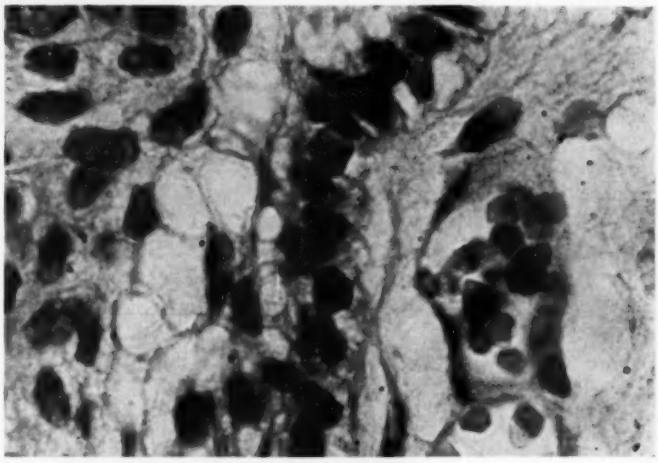

Fig. 2. — Higher magnification of the peripheral epithelial layer seen in Figure 1. The tumor cells abutting on the stroma are palisaded, cuboidal to columnar. Hematoxylin and eosin; reduced to 93% of mag. × 900.

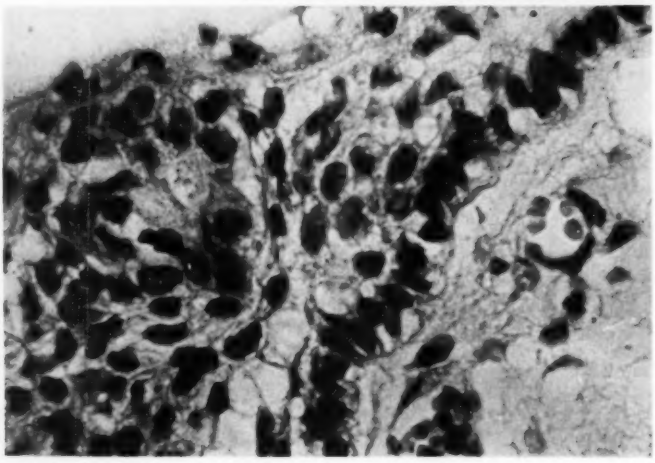

Fig. 3.—Loose arrangement of the more centrally located epithelial cells. Hematoxylin and eosin; reduced to 93% of mag. × 360.

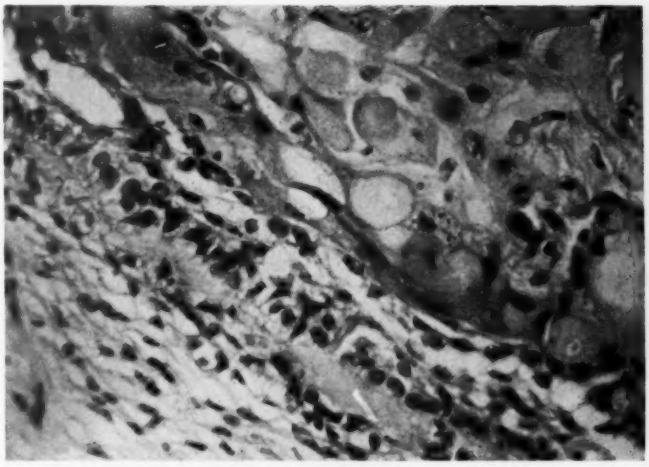

Fig. 4.—Appearance of eosinophilic "bodies" within the reticulated epithelium. Note the thickened basal membrane (suggestive of amelogenesis). Hematoxylin and eosin; reduced to 93% of mag. × 190.

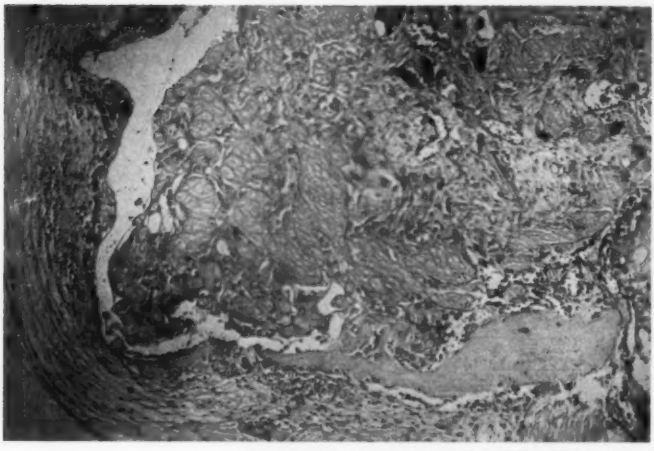

Fig. 5.—Aggregation of the eosinophilic "bodies" toward the center of the tumor. Hematoxylin and eosin; reduced to 92% of mag. × 40.

Fig. 6.—An aggregate of eosinophilic "bodies," showing its composition of "ghost epithelial cells" (photographed with the diaphragm of the microscope half-closed). Hematoxylin-eosin stain; duced to 92% of mag. × 360.

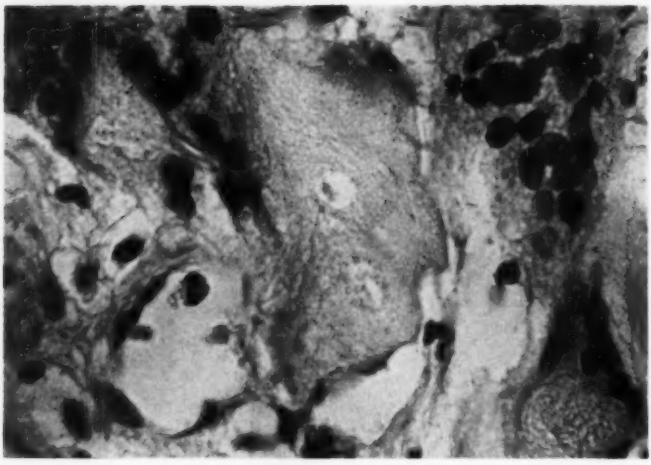

Fig. 7. — Photomicrograph from the center of Figure 5. Foreign-body giant cells engulfing "ghost cells" are seen. Hematoxylin and eosin; reduced to 92% of mag. × 360.

Fig. 8.—Staining of the "ghost cells" with the peracetic acid-azure-eosin method. The spared unstained areas correspond to the cell nuclei. Reduced to 92% of mag. × 190.

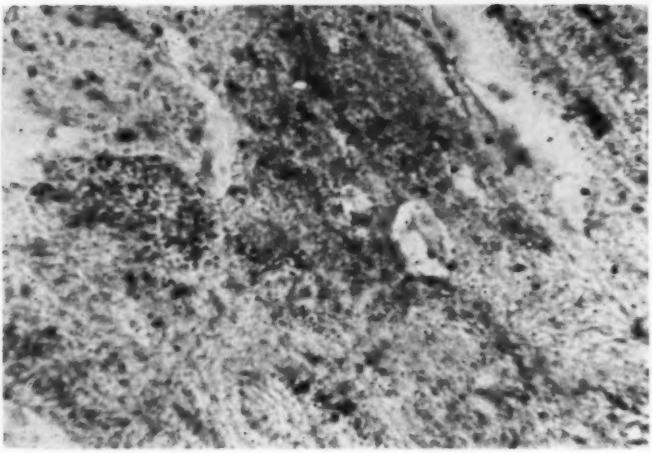

Fig. 9.—Fine calcium granules within the "ghost cells," revealed by the von Kossa stain. Reduced to 92% of mag. × 190.

the surrounding connective tissue in the form of small nests and thin strands (Fig. 1).

The special feature was the further differentiation of the neoplastic cells: Within the reticulated epithelium there appeared relatively large, round to oval, sharply demarcated, eosinophilic "bodies" (Fig. 4). Toward the center of the tumor aggregates of these "bodies" were formed (Fig. 5). On closer examination, especially with the diaphragm of the microscope half-closed, the eosinophilic "bodies" were found to be composed of a "ghost epithelium" (Fig. 6); i. e., they consisted of variously shaped, finely granulated and distinctly bordered clods, containing each the shadow of a nucleus. A granulation tissue with numerous foreign-body giant cells and variously sized, irregular, calcified masses (Fig. 1) were seen between the "ghost cells." Many of the giant cells were engulfing "ghost cells" (Fig. 7).

The question arose as to the meaning of the "ghost epithelium." We ventured to resolve this question with the aid of special techniques. With the peracetic acid-azure-eosin method [13] the "ghost cells" were colored a bluish-violet (Fig. 8), with sparing of unstained small, round areas, corresponding with the shadows of the nuclei. This reaction was not abolished by methylation. With the von Kossa stain a fine black granulation of the "ghost cells" was observed (Fig. 9). Other staining techniques did not help in establishing the nature of these cells and need not, therefore, be enumerated.

Comment

The diagnosis of ameloblastoma in the reported case is based on the characteristic features of this neoplasm: an epithelial tumor consisting of a peripheral layer of cuboidal to columnar, palisading cells, with central loosely arranged cells in a reticular pattern.

The appearance of "ghost cells," with and without calcification, in an ameloblastoma was mentioned by Thoma and Goldman [22] and by Robinson [19]; squamous-cell differentiation and keratinization of tumor cells are also known.[10,19] It is our impression, supported by the result of the peracetic acid-azure-eosin staining, that the "ghost cells" found in our case represent keratinized tumor cells. These cells behave biologically as foreign bodies. At one place there was a disruption of the outer epithelial lining, through which connective tissue from the surroundings invaded the interior of the tumor; there it was transformed into a foreign-body granulation tissue. The morphology of the keratinized cells and their dystrophic calcification make it apparent that we are dealing with a form of aberrant keratinization. This might be the reason for the foreign-body reaction.

In the series of Thoma and Goldman,[22] a tumor designated as an odontogenic mixed tumor (Case 52) is reported, of which a picture quite similar to ours is given; the authors described "ghost epithelial cells, which had undergone calcification." We do not think that our tumor is an odontogenic mixed tumor, as there is no second, mesenchymal, neoplastic element, and it is our opinion that the large calcified masses, occurring within the tumor, are completely calcified aggregates of "ghost cells." Prof. A. von Albertini,† who examined the specimen, concurred with our diagnosis and interpretation of the described "ghost cells" as an epithelial differentiation product. In this connection, it is noteworthy that Aisenberg,[1] in his classification of the ameloblastomas, described the varied contents of occurring cysts; he mentioned epithelial hyaline bodies and degenerating epithelium, simulating foam cells.

Pindborg and Weinmann [16] reported on two ameloblastomas, which were characterized by foci of "metaplastic transformation into a squamous epithelium with the formation of keratinized and parakeratotic pearls, which were calcified." They denied any

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similarity to enamel. The authors mentioned the well-known phenomenon of squamous metaplasia, followed by calcification in basal-cell carcinoma. They concluded that a relationship between the two kinds of tumors might exist. Pindborg,[17] under the heading of "calcifying epithelial odontogenic tumors," described three cases, in which the neoplasms were found in connection with embedded teeth. Histologically they consisted of epithelial sheets (not of ameloblast-like cells) invading the surrounding tissues. These tumors showed variously advanced stages of intracellular degeneration: The cells assumed a large circular form and were filled with a homogeneous substance, in which calcium was deposited. Although the tumors investigated by Pindborg and Weinmann [16] and by Pindborg [17] reveal some features reminiscent of our ameloblastoma, they are fundamentally different. We stressed the gradual transformation of the reticulated epithelium of an otherwise typical ameloblastoma into "ghost epithelial cells." Secondarily, calcium granules were deposited within these cells, and apparently they also provoked a foreign-body reaction.

We should like to draw attention to another tumor which exhibits features somewhat similar to those of the ameloblastoma described by us, namely, the calcifying epithelioma of Malherbe.[12] This tumor is composed of two types of cells: The first resembles the cells of a basal-cell epithelioma, and developing from it is the second type of cells, the so-called "shadow cells," which may be calcified. The "shadow cells" represent incompletely keratinized cells. In the stroma of the calcifying epithelioma foreign-body giant cells are found. Analyzing the description, the similarity between the calcifying epithelioma and the described variant of ameloblastoma (being a tumor of basal-cell character) suggests itself.

Summary

An ameloblastoma of the maxilla in a 13-year-old boy is described. The special feature of this tumor was the transformation of its cells into a kind of "ghost epithelium," which elicited a foreign-body reaction. It was shown histochemically that the "ghost epithelium" represented aberrantly keratinized and calcified cells. A certain resemblance of this tumor to the calcifying epithelioma of Malherbe is noted.

NOTE.—After the submission of this paper for publication, we found an article by Gorlin and Chaudhry, comparing the histologic features of ameloblastomas and craniopharyngiomas. The authors described in craniopharyngiomas the same histologic picture that we observed in an ameloblastoma, namely, a tendency to form metaplastic cornified "ghost epithelium," which became calcified; foreign-body giant cells were present at the edge of the "ghost epithelium."

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Observations on the Kidney After Phosphate Loading in the Rat

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The damaging effects of greatly increased dietary loads of phosphate ion on the kidney were summarized by MacKay and Oliver [1] in 1935. They showed that, though considerable variation in the toxicity of the various phosphate salts or of phosphoric acid could be elicited, the phosphate ion appeared to be essential. Of all the salts used, sodium phosphate N. F. (dibasic sodium phosphate; disodium hydrogen phosphate; Na_2HPO_4) was the most effective, and a load of 10% disodium phosphate in the diet would give easily demonstrable lesions within one day. In their animals at a 1% level of dietary phosphate, there was disorganization of the outer strip of the medulla, located chiefly in the terminal portions of the proximal convoluted tubule at the point of its diminution to join the thin descending loop of Henle. Over increasing periods of time, beyond the third day of loading, there was extension of the lesion to involve the more proximal portions of the proximal tubule. It was their opinion that there was no direct involvement of the thick or thin limbs of Henle, except by contiguity as these tubules passed through the involved area. McFarlane,[2] in 1941, found with heavier, more acute loads that lesions with crystalline deposits in the ascending limbs of Henle occurred.

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From The Children's Cancer Research Foundation and the Departments of Pathology, The Children's Medical Center and Harvard Medical School.

In this paper we wish to report observations on the kidneys of phosphate-loaded rats examined by histological and several histochemical methods, including microincineration, the Bunting procedure for the demonstration of phosphate ion,[3] and several procedures for the localization of oxidative and hydrolytic enzymes.

Materials and Methods

In these experiments male rats of the Sprague-Dawley strain, weighing from 80 to 150 gm., were divided into two main groups. The first were placed on a measured diet of ground Laboratory Chow to which was added 10% by weight of anhydrous disodium phosphate. They were maintained on this for periods of one to seven days, or were allowed to repair for four days on the control diet before being killed. Appropriate controls were pair-fed and killed simultaneously with their experimental pair-fed member. There were 22 animals in the group, 7 of these serving as controls.

The second group were prepared by starvation for two to three hours and then were given by stomach tube 5 cc. per 100 gm. of 10% solution of sodium phosphate N. F.; they were killed or died at intervals of 15 minutes to 7 hours after the loading dose. Two of the seven animals in this group also received a water load of 5 cc. per 100 gm. two hours before the phosphate load. Animals in this group were injected with alizarin subcutaneously one-half hour before loading.

All animals were killed by intraperitoneal injection of pentobarbital (Nembutal) sodium. Sections of the kidneys were taken, fixed in Zenker's solution without added acetic acid, cold acetone (−20 C), or cold 4% formalin (4 C), and quick-frozen in a bottle immersed in a mixture of carbon dioxide and alcohol. From the Zenker solution-fixed tissues, paraffin-embedded sections were prepared, and these were stained with toluidine blue and eosin as an oversight stain and the McManus periodic acid-leukofuchsin procedure. On the tissues which were quick-frozen the succinic dehydrogenase and Farber's TPN (triphosphopyridine nucleotide)-diaphorase stains were done. On the cold 4% formalin-fixed tissues, acid and alkaline

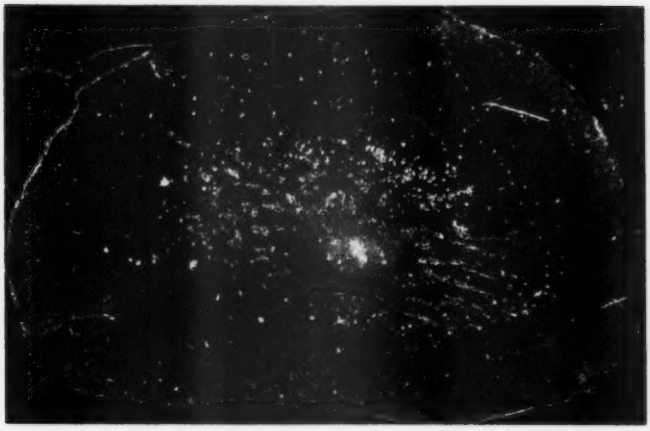


Fig. 1.—Ten per cent dietary phosphate load, one day. Kidney. Micro-incinerated specimen. Crystals are chiefly located at the corticomedullary junction and in the medulla. A few small crystals are present in the cortex. The papilla does not appear in the section. Dark field; reduced to 94% of mag. × 15.

phosphatase and esterase stains were done by the azo dye method, and Mallory's phosphotungstic acid hematoxylin mitochondrial stain and Sudan black stain for lipids were carried out. On the cold acetone-fixed tisues, acid and alkaline phosphatase by Gomori's cobalt-sulfide method, micro-incineration, alizarin-methyl green, von Kossa's stain for "calcium," and Bunting's stain for phosphates were done. In the chronically loaded animals routine histological examination was performed on the parathyroid, liver, stomach, lung, adrenals, and heart.

Results

Our results parallel in part those of MacKay and Oliver,[1] in that we found the major changes at the juncture of the cortex and medulla, and completely those of McFarlane.[2]

In animals killed after one day on the 10% phosphate-containing diet, minimal changes were detected in the terminal portion of the proximal tubules and in the

Fig. 2.—Control. Kidney. Esterase activity. Azo dye procedure; × 45.

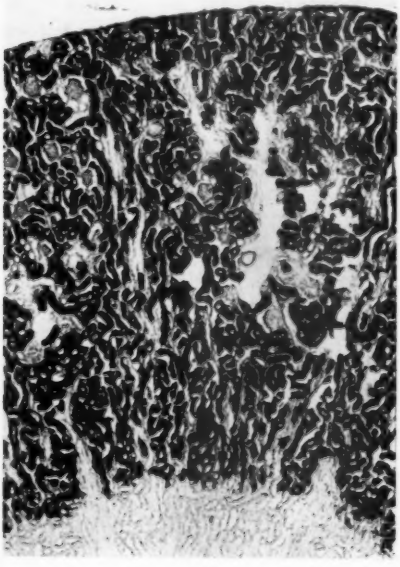
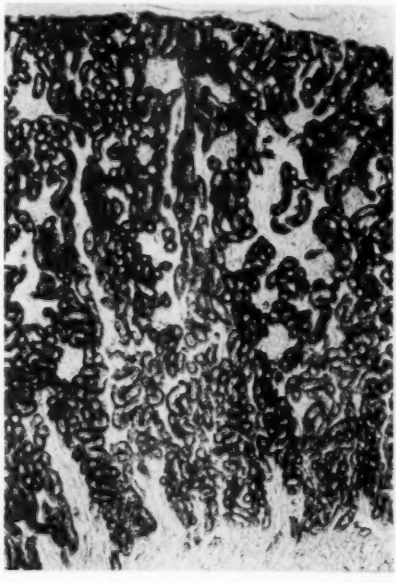


Fig. 3.—Ten per cent dietary phosphate load, one day. Kidney. A decrease in esterase activity is present in the inner cortex. Azo dye procedure; × 45.



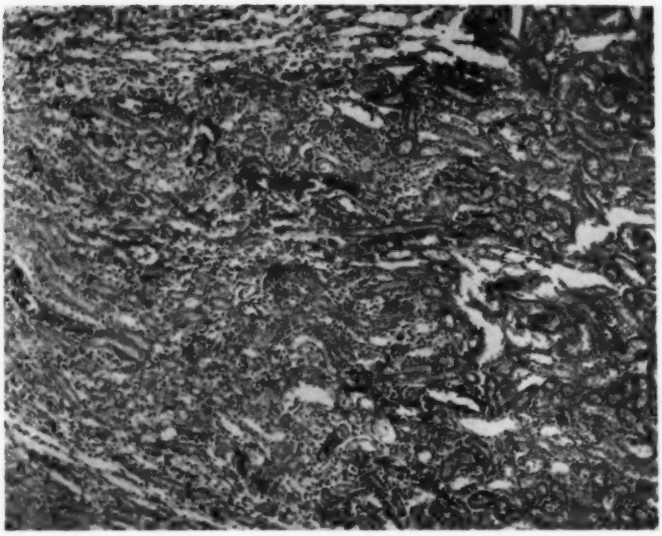


Fig. 4.—Ten per cent dietary phosphate load, two days. Kidney. Degeneration, inflammation, and disruption of the tubular pattern are seen at the corticomedullary junction. Toluidine blue and eosin; reduced to 83% of mag. × 95.

medulla (Fig. 1). Of the enzyme procedures only the nonspecific esterase was definitely depressed, (Figs. 2, 3).

After two days of feeding, the changes were much more marked. Now cytoplasmic basophilia could be recognized in some loops of the first portion and throughout the second portion of the proximal convoluted tubule, and the medulla. Casts appeared in the terminal portion of the proximal tubules, where dilatation, atrophy, and secondary inflammatory changes were present (Fig. 4). In the medulla, casts and calcium incrustation of necrotic cells were noted chiefly in the ascending limbs of the loops. In the micro-incinerated slides, the ash deposits were prominent at the inner border of the medulla nearest the papillae, but the heaviest deposits of mineral were at the junction of the cortex and medulla. The von Kossa stains gave an identical distribution. With the addition of strong sulfuric acid to a deparaffinized section, masses of monoclinic calcium sulfate crystals were formed on the solution of the original crystalline masses, indicating that calcium was a component of the original crystals. This same general localization appeared in the phosphate stain. The esterase and alkaline phosphatase enzyme (Figs. 5, 6) reactions had consistent reductions in staining at the corticomedullary junction. The localization of the crystals and the identification of the tubule affected could be seen quite clearly in the succinic dehydrogenase stains, where the ascending limbs strongly reduce formazan to give a purple color (Figs. 7, 8). In the loaded

Fig. 5.—Control. Kidney. Alkaline phosphatase. Cobalt-sulfide procedure; × 45.

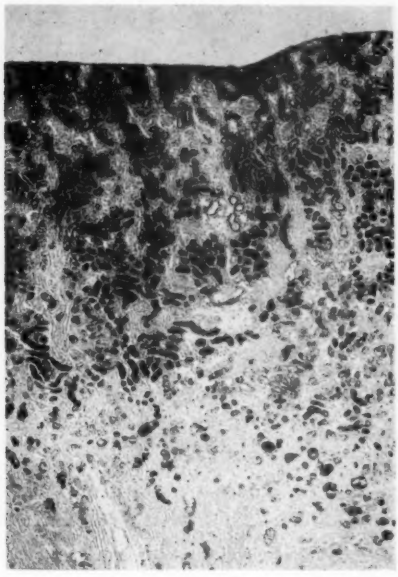


Fig. 6.—Ten per cent dietary phosphate load, two days. Kidney. A decrease in alkaline phosphatase activity of the inner cortex is present. Cobalt-sulfide procedure; × 45.

animals, deficiencies in staining reaction could be definitely identified in precisely those heavily stained ascending limbs that contained crystalline deposits (Fig. 9). Decreased staining was evident on the terminal portions of the proximal convoluted tubule as well, so that the whole corticomedullary junction had a decreased staining reaction.

After three days on the phosphate-supplemented diet, an accentuation of the features found in the tissues of animals fed for two days was present. In addition, there was now present dilatation of the proximal convoluted tubule secondary to obstruction at the corticomedullary junction, with peritubular inflammation in this region. Occasional deposits of crystalline material in the thin limbs of Henle's loop and the distal convoluted tubules, and evidence of regenerative mitotic activity in the terminal portion of the proximal convoluted tubule were also seen.

With a seven-day phosphate-fed animal there occurred an accentuation of the cast

Fig. 7.—Control. Kidney. Succinic dehydrogenase. × 45.

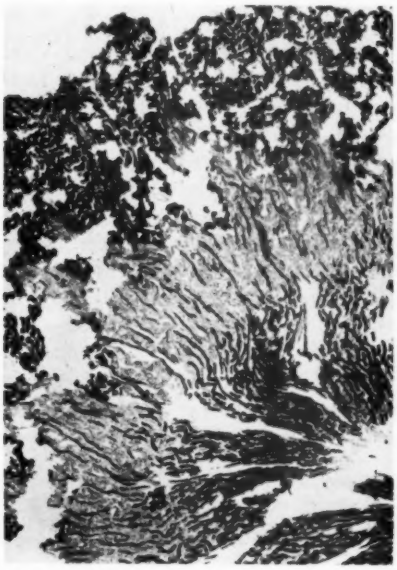
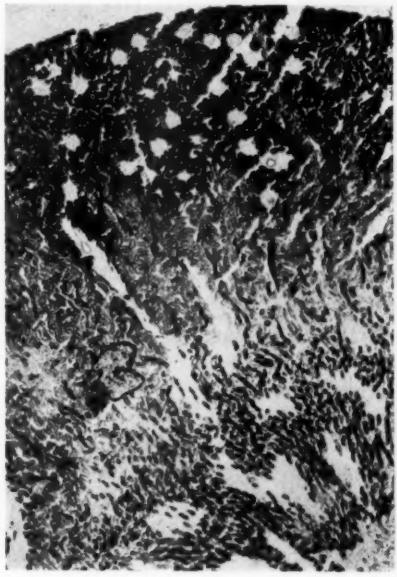


Fig. 8.—Ten per cent dietary phosphate load, two days. Kidney. The normally intensely stained thick limbs of Henle' loop show interruptions and decreased staining reactivity. Succinic dehydrogenase. × 45.



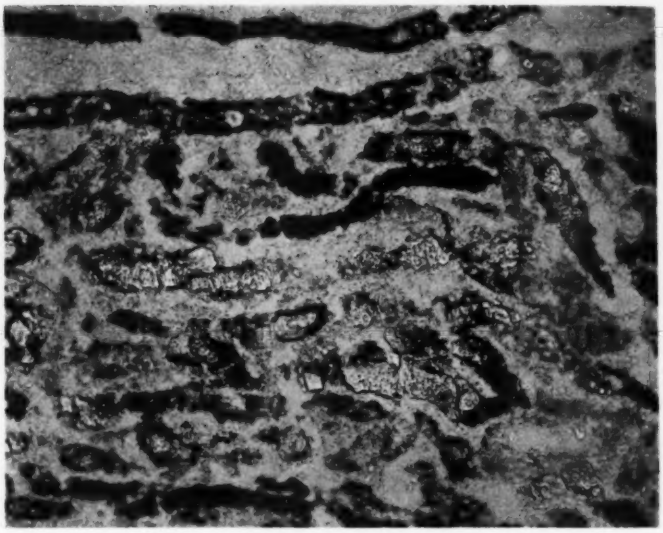


Fig. 9. — High-power magnification of Figure 8. The intimate relation of the crystalline masses to the broad limb is apparent. Reduced to 83% of mag. × 190.

formation in the distal convoluted tubule, the concretions now giving rise to segmental degenerations of tubular cells, distended tubules, and peritubular inflammatory reactions. The crystalline masses could be identified in both descending and ascending limbs of Henle's loop, and in the microincineration preparation the dense accumulation of these masses at the outer and inner borders of the medulla was particularly prominent.

In the group that was fed for three days with added phosphate and then allowed to subsist on the control diet for four days so that repair would occur, broad inflammatory scars ran through the outer cortex from the medulla to the capsule; the tubules in these areas were regenerating, and a large amount of crystalline material could be demonstrated in the microinceration preparation in the cortex, the juxtamedulla, and the medulla. In sections stained by the Bunting phosphate procedure the positive reaction was again limited to the terminal portions of the proximal convoluted tubule at the junction of the cortex and medulla. In the succinic dehydrogenase procedure the greatest deficiency in reactivity occurred at the outer extremity of the medulla, among the ascending limbs of the loop, where the majority showed interruptions in their continuity. Few of these appeared to pass into the cortex completely intact. The acid phosphatase and alkaline phosphatase reactions showed much restitution of pattern, but a significant defect remained in the alkaline phosphatase procedure in both the cortex and the corticomedullary junction, in comparison with the control stains. The changes toward restitution were less marked in the esterase stains.

In the animals fed the phosphate diet for one to seven days, no significant findings were encountered in the other viscera except for the presence of mitotic figures in the parathyroid glands, beginning in the animals fed for two days; the number of these varied from one in a cross section of a gland to several per high-power field. No evidence of tissue damage from metastatic calcification was seen, though no specific stains for phosphate deposits were carried out.*

The second group of animals, given 10% sodium phosphate by stomach tube, soon began to show severe clinical symptoms.

* Subsequent studies show the presence of mineral masses in the coronary vessels, where no histologic change on oversight stains could be recognized.

Distribution of Renal Lesions After Phosphate Loads

Period of Loading	Proximal Convoluted Tubule	Terminal Proximal Convoluted Tubule	Thin Limb of Henle	Thick Limb of Henle	Distal Convoluted Tubules	Collecting Tubule
1 day *	0	Cytoplasmic basophilia only	0	Intraluminal crystals	0	0
2 days *	Cytoplasmic basophilia	Cytoplasmic basophilia; crystalline casts; cell degeneration	0	Cytoplasmic basophilia; necrosis with encrustation and cast deposition	0	0
3-7 days *	Cytoplasmic basophilia; dilatation	Cytoplasmic basophilia; crystalline casts; cell degeneration, regeneration, inflammation	Occasional crystalline casts	Crystalline casts; cell necrosis; peritubular inflammation	Occasional crystalline casts	0
15 min.-7 hr. †	0	Intraluminal crystals	Crystalline casts	Crystalline casts; tubular necrosis	0	0

* 10% Na_2HPO_4 — dietary load.
† 5 cc/100 gm/10% NaH_2PO_4 by stomach tube.

These were hypothermia, tremors, extensor spasms of the hindlegs, paralysis, and shock. Diarrhea occurred in some. Grossly, at autopsy the stomach and intestine were seen to be distended with fluid; pallor was marked in the other viscera.

As early as 15 minutes after giving 5.0 cc. of 10% sodium phosphate per 10 gm. of rat by stomach tube, crystals could be found in the lumen of the terminal portions of the proximal convoluted tubules, but no certain alterations were seen in oversight, phosphate, or enzyme stains from the control.

By one-half hour after such a load, definite nuclear pyknosis could easily be seen in the medulla and in the ascending limbs of the loops of Henle, with some sloughing of necrotic cells into the tubular lumens. With the Bunting phosphate stain, positive-staining reaction at the upper border of the cortex and in the medulla was found; this, too, while not limited to the ascending limbs, was most marked in this unit. Crystalline masses within the tubular lumina were inconstantly present at the corticomedullary border. Of the enzyme stains, only the TPN-diaphorase activity was altered, with suggestive breaks in staining in the ascending limbs in the region of the pyknosis noted above. Four to six hours after such load, the pyknotic and degenerative changes were accompanied by crystalline masses, often rimming the tubular lumen of the terminal portion of the proximal convoluted tubule. Occasional precipitates involved the luminal border of the pyknotic tubular cells. Unlike other incrustations, these were PAS-positive, as were the necrotic cells of the ascending limbs (Fig. 10). Crystals could also be found in the thin limbs of Henle; but, unlike the broad limbs, no necrosis was evident (Fig. 11). The phosphate-staining reaction was limited to the medulla and corticomedullary junction, and usually was intense (Fig. 12). No constant changes were encountered in the enzyme stains, even though areas of nuclear pyknosis and apparent cell death were evident.

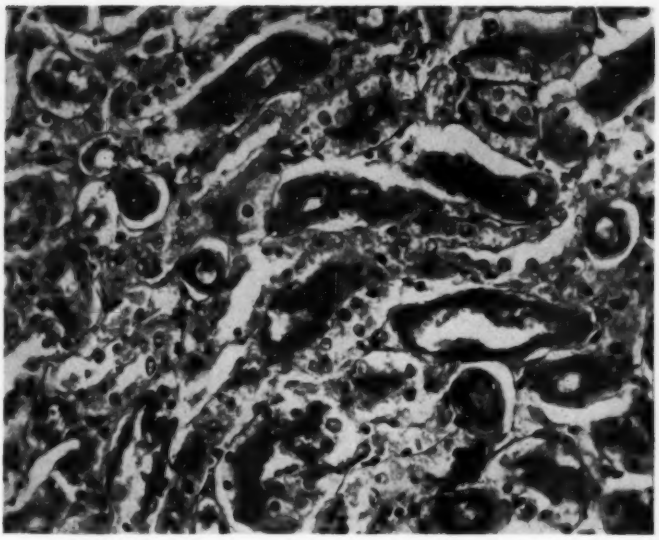


Fig. 10.—Acute phosphate load, four hours. Kidney. Intense degenerative changes are seen in the terminal portions of the proximal convoluted tubules, the cytoplasm giving a positive PAS reaction. Periodic acid-Schiff reaction; reduced to 83% of mag. × 325.

In none of these experiments was there any evidence of calyceal or pelvic formation of stones.

Comment

It seems clear from the results presented that phosphate loads, greater than the basic 1% level of MacKay and Oliver,[1] will directly damage other portions of the renal tubule than those incriminated by these authors. This damage, particularly, appears to be of importance at the higher dosage levels in the ascending limb of the proximal tubule. The use of oxidative enzyme succinic dehydrogenase and TPN-diaphorase stains is particularly valuable in locating these changes.

Of some importance to the understanding of the pathogenesis of the lesions described

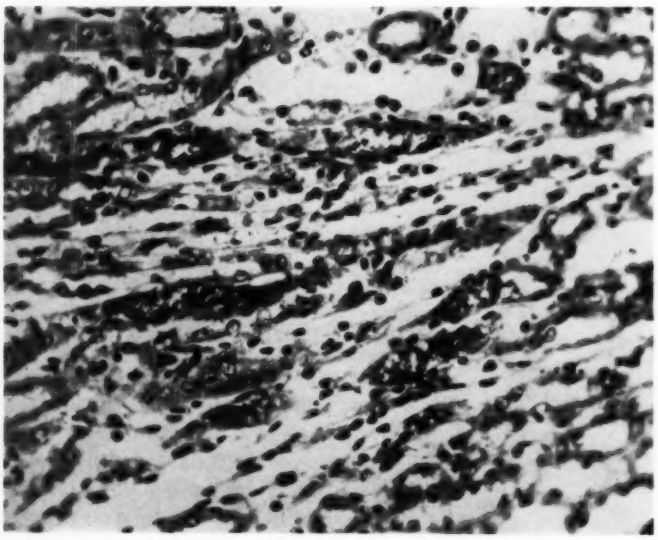


Fig. 11.—Acute phosphate load, four hours. Kidney. Intraluminal crystals and cellular degeneration are seen in the broad limbs in Henle's loop in the medulla. Toluidine blue and eosin; reduced to 83% of mag. × 275.

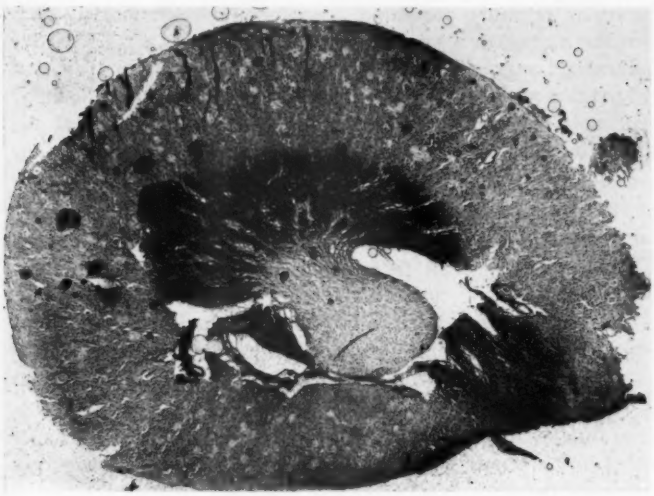


Fig. 12.—Acute phosphate load, four hours. Kidney. Intense staining is seen in the medulla and border of the inner cortex. Bunting phosphate procedure; × 15.

here is the fact that the deposits of mineral material, as determined by the von Kossa, alizarin, or microincineration technique, appeared always to be intraluminal and deposits in the cells occurred only when the cells became necrotic. In no instance were crystalline deposits found in any of the cells of the first portion of the proximal convoluted tubule, as was seen by Oliver [4] after exhibition of parathyroid hormone or vitamin D in spodograms of the dissected tubules. Since necrosis of tubular elements in the acute experiments appears to precede the deposition of crystalline material, and since the Bunting procedure does not strongly stain such crystalline material, the phosphate ions themselves must play a role in damaging the tubular cells in two areas most heavily affected. The formation of a white ash, as well as the abundant formation of monoclinic calcium sulfate crystals on exhibition of sulfuric acid to the ashed sections, indicates that the majority of the ashed material is indeed calcium phosphate.

The alterations in the enzyme activity are considered to be entirely secondary to damage to the tubular epithelium as a result of cast formation, secondary obstruction, pyelonephritis, or to the direct damage to tubular cells, with cell death. This is well exemplified again in the acute experiments where, although decided histological changes of cell death had occurred, no marked alteration in the hydrolytic or oxidative enzyme distribution could be found.

The quick proliferative response of the parathyroid over the one- to four-day periods of high phosphate loads may be expected from previous experiments on chronic phosphate loading, Crawford [5] having shown that parathyroid stimulation occurs within one hour after phosphate administration.

Are these findings helpful in localizing the point of resorption of phosphate from the tubule?

Under ordinary circumstances, at low serum phosphate values, phosphate is resorbed completely and at constant rate related to the glomerular filtration rate, phosphate presented in the tubule over this maximum being excreted.[6] At ordinary dietary phosphate loads tubular resorption of phosphate is almost complete. At least in Necturus, there is no evidence of tubular secretion of phosphate,[7] although this has been shown to occur in the chicken.[8] As serum phosphorus rises, parathormone activity lowers the maximum rate of phosphate resorption, and this may reach a level where virtually no resorption occurs.[5] Increases in phosphate excretion above this level will then depend upon increasing serum

levels or increases in glomerular filtration of the phosphate.[5] All inorganic phosphate is considered to be ultrafiltrable,[9] and in Necturus [7] it has been suggested that calcium phosphate may pass the glomerulus as a complex and not be secreted as an ion.

The signs of tetany in our animals in the acute experiments suggest that serum calcium ionization was probably decreased, as a result of the formation of calcium phosphate complexes in the serum.

The location of the portion of the tubule involved in resorption of phosphate is primarily derived from inference and data on amphibia. In amphibia the first one-quarter of the proximal tubule shows little evidence of phosphate concentration; this becomes considerable in the more distal portions, and here is considered to be due to water resorption. In such organisms the greatest concentration in the tubular lumen is reached in the distal tubule; yet with low plasma values the bladder urine may contain no phosphate.[7,9] Resorption must also take place in this area as well. These data are consistent with the finding of Ullrich [10] that the concentration of inorganic phosphate is low in the cortex. The absence in our experiments of stainable phosphate in the outer cortex, containing the greatest portion of the proximal tube, is also pertinent.

If, as suggested, phosphate passes the glomerulus as a calcium phosphate complex and little phosphate is resorbed in the proximal tubule, the precipitation in the terminal portions of the proximal tubule of calcium phosphate may parallel the increasing resorption of sodium and water along the tubule; thus, the volume of fluid to maintain the solubility of calcium phosphate will become insufficient at high loads, leading to precipitation of calcium phosphate.

The phosphate loads given here will tend to act as an osmotic diuretic. This then will tend to decrease the resorption of water in the proximal and distal tubules. In face of this, however, particularly in the acute experiments, there is marked dehydration due to losses of fluid from the plasma into the intestine. This tissue dehydration will elicit an antidiuretic response. As a result, active resorption of fluid will occur in the distal tubule. Such further resorption of fluid may account for the crystal deposition in the distal convoluted tubules in the cortex on the basis of removal of water from an already saturated solution.

If, as Wirz believes,[11] redilution occurs in the ascending limb of Henle and final adjustment of osmolarity does not occur until the lower medulla is again reached, the precipitation of the crystals in the medullary portions of the ascending limb is difficult to explain.

The alternate explanation of Berliner et al.,[12] that the decreased osmolarity in the ascending limb and cortical portion of the distal tubule, as determined by puncture techniques, is achieved by active sodium transport out of the tubule, may provide an explanation for the precipitation of calcium phosphate in these areas. It may be that, by the removal of sodium ion, the solubility of the remaining phosphate will be reduced, according to the common ion effect.

The absence of significant crystal deposition in the papillae and the failure to have distinct concentration in the innermost zone of the medulla, since concentrations in general of the urine solutes tend to rise from cortex to papillae,[13] does not fit Wirz' thesis. The only suggestion is that in the final adjustment of urinary pH the urine is made sufficiently acid to increase the solubility of the phosphate so as to prevent precipitation here, even though concentration of urine may increase further over that in the distal convoluted tubule. In experimental citrate loading,[14] acetazolamide (Diamox) will cause precipitation of calcium citrate in just this area, presumably owing to the blockage of hydrogen ion exchange and failure of acidification of the urine. Preliminary experiments with added acetazolamide did not result in papillary precipitation in subacute loading experiments.

Conclusions

The loading of sodium phosphate N. F. (disodium phosphate) in rats via the gastrointestinal tract over periods of 15 minutes to 4 days results in deposition of calcium deposits in renal tubules and in the necrosis of renal tubular epithelial cells in the terminal portion of the proximal tubule and the ascending limb of Henle and, during longer-loading periods, in the distal convoluted tubule. The first portion of the proximal tubule, as well as the collecting tubule, is spared. In view of the physiology of phosphate resorption and excretion, the ascending limb of Henle is suggested as the main area for phosphate resorption.

The Children's Hospital.

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Effect of Age and Heat on Human Collagenous Tissue

Studies on Acid Solubility, Titration Curves, and Elasticity

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Previous studies based on the swelling properties of human tendon have shown that, with increasing age, such tissue passes through several stages of increasing rigidity.[1,2] A similar increase in rigidity with age has been demonstrated in perivascular connective tissue and myocardium.[3,4] Heat treatment caused a decrease in swelling ability of tendon and myocardium, and thermodynamic calculations were consistent with the view that increasing rigidity of collagenous tissue was due to thermal denaturation at body temperature.

In order to explore further the possibility that aging of collagen depends on thermal denaturation, it was decided to compare the effects of age and heat on certain other properties of collagenous tissue. The greater the extent to which the aging changes in collagen are identical with those that may be produced by heat, the greater the likelihood that the former are due to thermal denaturation. The present study was undertaken in order to compare the effects of age and heat on acid solubility, acid- and base-binding capacity, and elasticity of human tendon. It was also anticipated that data acquired in this investigation might provide information on the types of molecular changes which occur in aging of collagen.

Methods

Collagen investigated was from the central tendon of the human diaphragm. Tendons were obtained from autopsy cases in which the diaphragm was not directly involved in any disease process. Loose tissue was scraped from the pleural and peritoneal surfaces so that only the meshwork of collagen fibers remained. This meshwork was then stored at —60 C until used.

Acid solubility of collagen was determined by maintaining tendon in 0.05% acetic acid adjusted to pH 2.4 with hydrochloric acid. Each tendon was minced and added to 100 vol. of the acid. The mixture was agitated at room temperature for 24 hours, and the supernatant fluid was aspirated. An additional 100 vol. of acid was added and the process continued for another 24 hours, after which the insoluble material was filtered off and washed briefly on the paper with additional acid. More prolonged extraction did not result in additional collagen going into solution. Collagen content of the insoluble residue and of untreated, control fragments of the same tendon was determined by the hydroxyproline method of Neuman and Logan.[5] The hydroxyproline determined was multiplied by 7.35 to obtain the amount of collagen, which was expressed as percentage by weight of fresh tendon. Subtracting the percentage collagen remaining after extraction from percentage collagen in the untreated tendon gave the amount of collagen which dissolved in the acid. This quantity which went into solution was expressed as percentage of original collagen.

To determine acid and base binding as a function of age, titration curves of old and young tendons were studied. Tendons were finely sliced while frozen, minced with scissors, and finally ground while cold to a homogeneous suspension in a ground-glass homogenizer. Homogenates were diluted to yield approximately a 0.2% solution of collagen. The diluent for acid titration was 0.1 M NaCl; for alkaline titration, 0.1 M Na_2SO_4. These salts were used to suppress swelling of the collagen during titration.[6] Standard hydrochloric acid and sodium hydroxide, made to the same salt concentration as the collagen suspensions, were added to the collagen suspensions. The pH values were determined with a glass electrode pH-meter equipped with a special electrode for high pH measurements. Blank salt solutions were also titrated.

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U. S. Public Health Service Fellow in Pathology (Dr. Kohn).

The pH values of the collagen suspensions and blanks were plotted as a function of the milliequivalents of acid and base added. At every pH value the amount of acid or base added to the blank was subtracted from that added to the collagen. This gave the amount fixed by the collagenous tissue at each pH. Aliquots of the suspension were removed and the collagen content determined. The amount of acid and base fixed per gram of collagen was then determined as a function of pH.

Elasticity was studied using single bundles of parallel tendon fibers. Tendons were placed in 0.9% NaCl and the individual bundles, about 0.2 mm. in diameter and 15 mm. long, were obtained by teasing the softened tendon. Excess water was removed from the fibers by blotting with filter paper, and the diameter of each fiber bundle was measured with an ocular micrometer. Cross-sectional area was calculated from the value for diameter. One end of the bundle was then placed in a clamp attached to the left end of the arm of an analytic balance. The lower end of the bundle was held by a clamp which could be moved vertically by a rack and pinion. As weights were added to the right side of the balance, the clamp holding the lower end of the fiber bundle was lowered until the balance pointer was at the zero position, indicating that the same tension was exerted on the bundle as was exerted by the weights in the right balance pan. Length of the fiber bundle as weights were applied was determined with a cathetometer. Bundles were moistened with isotonic saline during these measurements.

At each weight applied, weight exerted per millimeter of cross-sectional area of the fiber bundle was calculated. This value represented the stress exerted. The fraction of the original length which the fiber lengthened as stress was applied represented the strain. The ratio of stress to strain yielded the coefficient of elasticity, or Young's modulus. The stress at which breaking of the fiber bundle occured was also noted.

Results

Acid solubility was studied in 10 tendons of a young group comprised of persons between 1 and 30 years of age, and in 11 tendons from an old group, in which the members were between 54 and 80 years of age. Duplicate determinations of each tendon were made, with values that were within ±5% of the mean. Results are summarized in Table 1. The greatest acid solubility is in the very young tendons, but as a group the young tendons contain a percentage of soluble collagen which is significantly greater than that contained in the old tendons.

TABLE 1.—*Percentage of Collagen in Each Tendon Which is Soluble in Acid* *

Age, Yr.	% Soluble Collagen
Young Group	
1	93
1	93
2	62
3	27
4	19
10	37
15	36
27	39
28	0
30	24
Old Group	
54	25
58	5
59	8
62	0
65	9
68	13
69	14
70	35
73	13
78	6
80	27

* For difference between young and old, $P<0.01$.

The effect of heat on the acid solubility of collagen was tested in six tendons from persons between 3 months and 16 years of age. Tendons were heated in mammalian Ringer's solution at 56 C for various

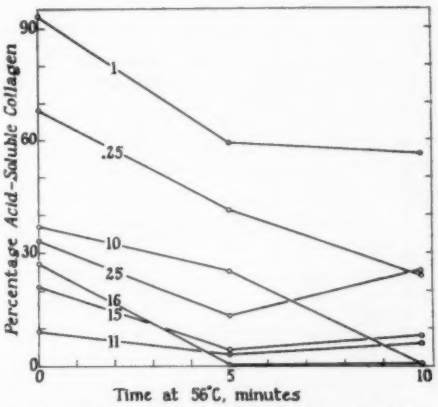

Chart 1.—Effect of heating tendon at 56 C on percentage of collagen which is subsequently dissolved by dilute acid. Age of tendon in years is indicated on each curve.

lengths of time and cooled to room temperature, after which percentage of soluble collagen was determined. As shown in Chart 1, heat treatment consistently decreases the amount of collagen which is soluble in acid. No collagen was dissolved as a result of heat treatment alone.

In studying the acid- and base-binding capacity of tendon, titration curves of a number of young and old tendons were determined. Typical curves are shown in Chart 2. The general shape of the curves does not vary with age. However, differences in acid and base binding at low and high pH levels are apparent. In a study of eight young and eight old tendons, no consistent age-related variation in acid binding could be demonstrated, while there did appear to be greater fixation of base by young tendon than by old. Therefore, 15 young tendons from persons between 1 and 28 years of age were compared with 15 old tendons from persons between 52 and 92 years of age in regard to the amount of base fixed at pH 10. Data from these determinations are shown in Table 2, and indicate that significantly greater base is fixed by young tendon than by old.

The effect of heat was studied in 10 tendons selected from the young group. Intact tendon fragments were heated in mammalian Ringer's solution for 15 minutes at 56 C and cooled to room temperature. They were then homogenized, as were the unheated, control fragments from adjacent tendon, and base-fixing capacity was determined. Results are included in Table 2 and show that heat treatment has a significant effect in decreasing the amount of base fixed by tendon at pH 10.

The elastic properties were investigated in 10 tendons from a young group 8 to 30 years of age and 10 tendons from an old group, the members of which were between 68 and 94 years of age. Very young tendons were not used because the fiber bundles were too fine and the weave too dense to yield satisfactory specimens. Three fiber bundles from each tendon were studied, with the exception of one old and one young

TABLE 2.—*Milliequivalents of NaOH Fixed per Gram of Collagen at pH 10*

Age, Yr.	Milliequivalents Fixed *	Milliequivalents Fixed After Heating †
	Young Group	
1	1.56	1.56
2	1.27	1.48
3	1.89	1.28
4	1.95	1.89
6	1.37	1.26
7	1.54	0.84
10	2.11	1.34
12	1.56	
15	1.69	1.64
16	1.84	
21	2.51	1.59
22	1.40	
22	1.14	
27	1.60	
28	1.63	1.36
	Old Group	
52	1.52	
56	1.47	
57	1.54	
59	1.77	
60	1.71	
63	1.28	
63	1.20	
67	0.97	
69	0.89	
70	1.64	
72	1.26	
73	1.45	
78	1.81	
82	1.50	
92	0.92	

* For difference between young and old, $P<0.05$.

† Heated at 56 C for 15 minutes. For comparison with nonheated specimens by sign test, $P<0.05$.

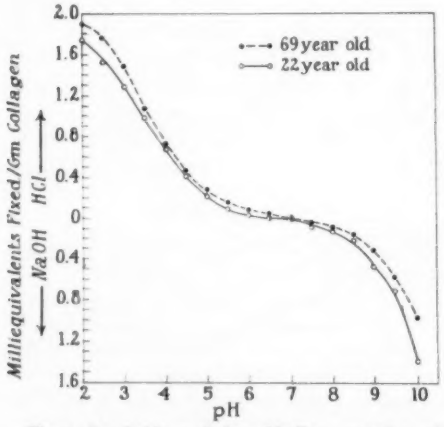

Chart 2.—Acid- and base-binding capacity of tendon as a function of the pH.

tendon, from which only two adequate bundles could be obtained.

Stress-strain plots were constructed for each fiber bundle. After stresses above 0.5-1.0 kg/cu. mm. were applied, the plots became linear and remained so until the elastic limit was reached. This limit was extremely close to the breaking stress. Young's modulus was determined for the linear part of the plots. Analysis of variance of Young's modulus and breaking stress indicated that within each group, young and old, there was no greater variation between bundles from different tendons than between bundles from the same tendon. It therefore seemed justifiable to consider each determination separately rather than calculate a mean value for each tendon.

Values for Young's modulus and breaking stress for all bundles within both young and old groups are plotted in Chart 3. The number of bundles within each group showing the value of the abscissa are plotted on the ordinate. There appears to be a slightly but significantly greater Young's modulus and stress required for breaking in bundles from young tendons than for bundles from old tendons. Because of the great overlap in distributions of young and old, it was believed that a study of the effect of heat would yield equivocal results. Therefore, such a study was not attempted.

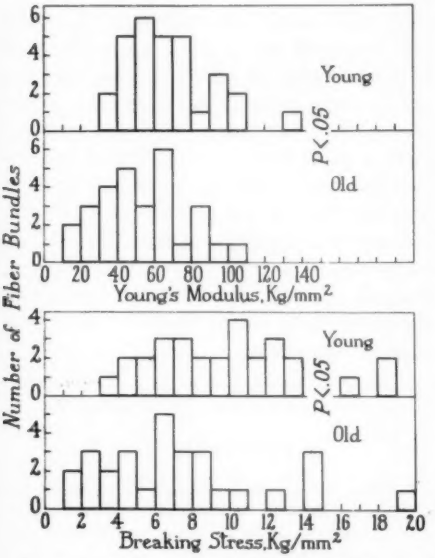

Chart 3.—Distributions of Young's modulus and breaking stress of collagen fiber bundles from young and old subjects. Numbers of bundles which gave the values of the abcissa are plotted on the ordinate.

Comment

Acid solubility of collagen as a function of age has been studied in human skin and Achilles tendon by Banfield.[7] His observations and those of this investigation are in accord to the extent that greatest solubility was found to occur in infants. However, Banfield found little or no difference in solubility between old material and younger collagen from persons over 5 years of age in the case of Achilles tendon and over 1 year of age in the case of skin. Banfield evaluated the amount of soluble collagen by the turbidity noted on neutralization of the solution. That method is less sensitive than the chemical determination used in the present investigation and may explain why an age difference in solubility past infancy was not observed.

Loss of solubility with age might be due to an aggregation of polypeptide chains or fibrils of collagen, resulting in the formation of larger particles, which are not dispersed in acid. The similar loss of acid solubility following heating could be explained by the same mechanism, as it is known that thermal denaturation of proteins causes an aggregation of molecules with an increase in particle weight.[8]

Several mechanisms may be hypothesized for the loss of base-fixing capacity noted with increased age. One possibility is a decrease in number of acidic groups, although there is no evidence that the composition of collagen changes with age. The possible role of acid polysaccharides in base binding must also be considered. Sobel and Marmorston have observed a decrease in the gel-fiber ratio in skin connective tissue with age,[9] and such a change might influence base binding. It was shown in a previous

study of diaphragm tendon, however, that concentrations of water and collagen, which together comprise about 90% of the tendon weight, do not vary with age. Thus, any change in polysaccharide concentration with age in this tissue would be very slight. A third possible mechanism may be aggregation of polypeptide chains with age, as proposed to explain loss of acid solubility. Such aggregation might mask acid groups, making them inaccessible for titration. Heat would have a similar effect in causing aggregation.

The slight decrease in Young's modulus and in breaking stress with age probably results from a change in the number, direction, and strength of intra- and intermolecular bonds of collagen. A more specific explanation cannot be made, because the strength and elasticity of a collagen bundle depend on bonds parallel to the long axis, as well as on lateral bonds, which prevent molecules and fibers from slipping past each other. This study does serve, however, to point out that change in elasticity of collagenous tissue with age does not depend on elastic properties of fibers as determined in one dimension parallel to their long axes.

Instead, the important age-related change would be a marked increase in bulk modulus. This was indicated in the previous investigation, in which a prominent loss of osmotic swelling ability of collagen was observed with advancing age.[1] Swelling of collagen under the conditions of that study is inversely proportional to the bulk modulus.[10] Such an increase in bulk modulus with age indicates that as tissue which contains appreciable collagen ages, it will become more rigid and offer greater resistance to deforming forces.

Increase in bulk modulus can probably best be explained on the basis of an increase in strength and number of lateral bonds among polypeptide chains of the collagen molecule. Changes in acid solubility and base-binding capacity described in the present report could also be due to increased lateral binding, with consequent aggregation of peptide chains.

The observation that heat treatment causes changes similar to aging in swelling ability, acid solubility, and base-binding capacity supports the view that aging of collagen may be due to thermal denaturation. Additional support for this view is provided by the finding that both heat and aging increase the calcium-binding capacity of rat tendon.[11] Although heat causes age-like changes to occur, the reaction is not necessarily of first order. Physiological substances, which act as tanning agents, may participate in the formation of lateral bonds, as proposed by Bjorksten.[12] This possibility is under investigation.

Summary

Studies on the effect of age and heat on collagenous tissue in regard to the properties of acid solubility, acid- and base-binding capacity, Young's modulus, and breaking stress were undertaken. Tissue investigated was the central tendon of the human diaphragm. Both heat treatment and age resulted in a decrease in percentage of the collagen which was soluble in dilute acetic acid at pH 2.4. Similarly, heat and age decreased the base-binding capacity of tendon at pH 10, although changes with age in shape of titration curves and in acid binding were not observed. There appeared to be a slight decrease with age in Young's modulus and breaking stress of collagen fiber bundles. It was pointed out that these and previous findings indicate that collagenous tissue becomes more rigid with increasing age, and that such an age-related change might be due to thermal denaturation at body temperature.

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Disseminated Demyelination of the Brain Following Co^{60} (Gamma) Radiation

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The effects of ionizing radiation upon the central nervous system have been extensively studied. Early and late morphological changes are generally recognized. Many observers believe that blood vessels bear the brunt of the irradiation; others, however, favor a primary damage to the neurons and the glial cells.

Early primary neuronal damage has been described in animal brains following single doses of very high energy rays. Ellinger [1] showed that roentgen rays at doses of 10,000 r destroyed nerve cells in the medulla of the goldfish. Hicks et al.[2] saw alterations in the granule cells of the cerebellum of mice exposed to 10,000 r of roentgen rays, and at higher doses necrosis of the forebrain neurons became evident. They also described acute patchy oligodendromyelin necrosis in the forebrain after exposure to cathode rays. Bering et al.[3] studied the effects of localized irradiation from radioactive tantalum (Ta^{182}) implants on monkey brains and found that there was more damage to the neurons than could be accounted for by mere vascular obliteration. Haymaker et al.[4] showed that total-body exposure of monkeys to radiation from a Ba^{140}-La^{140} source at doses below 10,000 r produced a vasculitis in the cerebrum and meninges within several hours. By contrast, no inflammatory reaction of the blood vessels was found in the thoracic or abdominal viscera, in spite of the same exposure to the radiation. With doses above 10,000 r, pyknosis of the granule cells of the cerebellum appeared, in addition to the vascular changes. These experiments were confirmed by Vogel et al.,[5] using Co^{60}.

Delayed effects, sometimes manifesting themselves as necrosis years after irradiation, have been described in man after treatment for extra- or intracranial tumors (Fischer and Holfelder,[6] Brandenburg and Maurer,[7] Kalbfleisch,[8] Pennybaker and Russell,[9] and Boden [10]). This delayed radionecrosis of the brain was related by these authors to the severe degenerative and obliterative alterations of the small blood vessels which were always present. These vascular changes, well illustrated by Courville and Myers,[11] range from hyaline, amyloid, vacuolar, and fatty degeneration to fibrinoid necrosis, thrombosis, and perivascular hemorrhage. Russell et al.,[12] who studied delayed radionecrosis of the brain in rabbits, were less definite regarding the pathogenesis of these lesions. About 100 days after a single dose of 2,850 r of x-rays, the animals developed neurological signs. The lesions consisted of foci of hemorrhage and necrosis, which appeared to be related to the capillaries. Increased permeability and fibrinoid necrosis of the vessel walls accompanied the parenchymal lesions but did not precede them. A rabbit killed at 90 days after irradiation presented considerable focal degeneration of the Purkinje cells, a few cortical hemorrhages, but no vascular abnormality. Progressive degenerative and obliterative changes of the vessels, as well as pronounced gliosis, were striking only in the more advanced stages.

The purpose of our paper is to present the histopathology of a case of radionecro-

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sis of the brain occurring about three months after a course of Co^{60} γ-radiation for basal-cell carcinoma of the left ear. The presence of punched-out areas of demyelination with only slight damage to the nerve cells and axons in the early lesions, the intensity of the widespread vasculitis, the abundance of plasma cells, and the presence of giant cells differ from other reported cases of delayed radionecrosis, in which more emphasis has been laid upon the degenerative and obliterative alterations of the blood vessels.

Report of a Case

In March, 1953, a 32-year-old housewife had a basal-cell carcinoma removed from her left external auditory canal. Postoperatively the external canal was treated by means of a radium applicator, a surface dose of 1,000 r being delivered in a period of 45 minutes. For three years after the original operation the ear remained normal. She then had pain and discharge from the ear, and a fleshy mass was seen to be filling in the canal. A left radical mastoidectomy was performed in February, 1956. Histological examination of the specimen showed recurrent basal-cell carcinoma.

In August, 1957, a large recurrent mass was again treated in the external auditory canal, the tumor having spread to involve the tragus and preauricular region. It was estimated that the deep extension of the tumor was not less than 3 cm. from the surface of the tragus. Radiological examination was noncontributory because the previous mastoidectomy scar obscured the picture. No biopsy specimen was taken on this occasion, because the clinical appearances were typical and it was felt that further operative intervention might reduce the radiosensitivity.

From Aug. 29 to Sept. 26, 1957, the patient was treated with Co^{60} radiation by means of a single field 6×6 cm. directly over the left ear. A tumor dose of 5,000 r in a period of four weeks was considered safe and, at the same time, tumor-lethal. The depth of the tumor was considered to be 3 cm. (87% depth dose). Physical factors: H. V. L., 11 mm. Pb; dose rate, 34.97 r/mm.; 20 treatments in 29 days; daily dose 288 r, to a given maximum of 5,750 r (100%) (Fig. 1). On Dec. 12, 1957, the patient was admitted to the Toronto General Hospital with a two-week history of blurred vision, followed by diplopia, nausea, and vomiting. The patient also complained of slurred speech and difficulty in walking and swallowing.

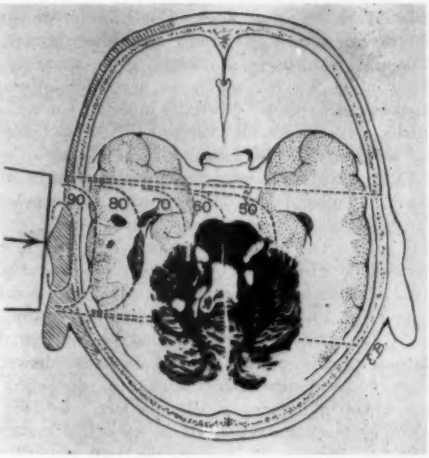


Fig. 1.—Disseminated plaques of demyelination involving the gray and white matter of the temporal lobe and the cerebellum are apparent within the beam of Co^{60}. The lesions of the left temporal lobe were not found on the same horizontal plane as that represented by the drawing. Only a small rim of subependymal tissue was involved in the right temporal lobe. The figures represent the depth-dose percentages. Celloidin-embedded; stained with Loyez' method for myelin sheaths.

Physical Examination

The patient was drowsy; her speech was dysarthric and dysphasic. There was coarse bilateral horizontal nystagmus. The corneal reflexes were decreased. There was profound cerebellar ataxia with gross impairment of rate, range, and force of movement bilaterally, slightly greater on the left. Upward extension of the toes was present bilaterally. No sensory defect or papilledema was found. The laboratory findings were noncontributory.

Course

Recurrent tumor within the posterior cranial fossa was considered to be the most probable explanation for the clinical state. Accordingly, on Dec. 17, a positive contrast ventriculogram (iophendylate [Myodil]) was made, but this showed no abnormality. The same day the patient became akinetic and mute. She remained in decerebrate rigidity until her death, on Dec. 27, 1957. The clinical diagnosis was acute disseminated demyelinating disease involving the brain stem, possibly related to the radiotherapy.

Postmortem Findings

Gross Examination

The brain weighed 1,225 gm. Some subdural and subarachnoid hemorrhages were found in

relation to the burr holes. The convolutions of the vertex were well formed and closely packed. The cerebral arteries showed no gross abnormalities. There was no significant herniation of the hippocampal unci or cerebellar tonsils. The ventricles were moderately narrowed. Several translucent grayish plaques, the largest measuring 0.5×1.5 cm., were noted in the white matter of the left temporal lobe, confined to a region overlying the left auditory canal and middle ear (Fig. 1). The pole of the left temporal lobe showed no gross changes. A small subependymal area of softening was present in the right temporal lobe (Fig. 1). Sections of the midbrain revealed no gross areas of demyelination. A horizontal section of the cerebellum and midpons showed numerous well-demarcated plaques of demyelination involving predominantly the left side (Fig. 1). The plaques were noted in the white matter and the dentate nucleus of the left cerebellar hemisphere and in both middle cerebellar peduncles. There was also involvement of the subependymal tissue of the floor of the fourth ventricle. Sections of the lower pons presented confluent areas of translucent grayish tissue, involving mainly the posterior and lateral portions of the left middle cerebellar peduncle. The open medulla presented only a peripheral rim of normal tissue, greater on the right side. The gray and white matter were indistinct. The pyramid and the inferior olivary nucleus were involved on both sides. This area of demyelination was continuous throughout the medulla, but in the lower medulla the lesion was confined to its left dorsolateral area. The cervical and thoracic cord showed no abnormalities. The lumbar enlargement contained a small hydromyelic cavity.

Microscopic Findings

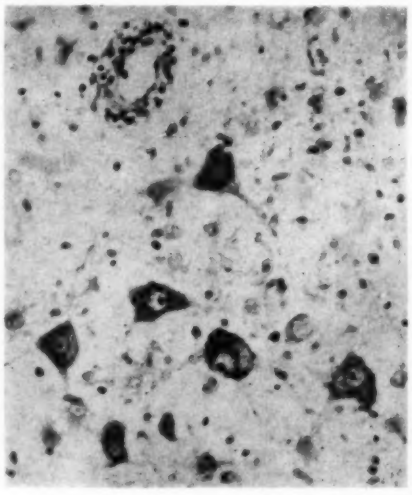
The tissue from the left external auditory meatus and the bone underlying the irradiated field contained no tumor, and the bone was viable. Blocks of the brain were taken from regions within the beam of the Co^{60} radiation and compared with blocks distant from it, such as of the frontal lobes and spinal cord below the medulla oblongata. The sections were stained with hematoxylin and eosin, cresyl violet, phosphotungstic acid hematoxylin, Mallory's connective tissue stain, Laidlaw's reticulin stain, Smith and Quigley's myelin stain, Bodian's axon stain, Ramón y Cajal's gold-sublimate method for astrocytes, Cone and Penfield's silver carbonate method for microglia and oligodendroglia, and oil red O and scarlet R for neutral fat.

The areas of demyelination and necrosis were limited to the tissues which had been within the beam of the Co^{60} radiation. Besides the grossly described punched-out plaques, smaller areas of demyelination were seen in the left temporal lobe, the dorsolateral portion of the left thalamus, and lateral to the left substantia nigra in the midbrain. Slight infiltration of chronic inflammatory cells was noticed in the leptomeninges over a much wider area. Similarly, slight perivascular cuffing with lymphocytes was noted in nonirradiated brain and cord tissue. The following alterations of the nervous and mesenchymal tissues were found in the irradiated portions of the brain.

Neurons

The Purkinje cells were reduced in number, and some swollen, degenerative forms were present. The nerve cells of the cerebral cortex showed no definite changes. Plaques of demyelination extended into the granular layer and the dentate nucleus of the cerebellum on the left side. The inferior olivary nucleus and some of the nuclei of the cranial nerves in the medulla were involved on both sides. A remarkable preser-

Fig. 2.—Well-preserved cells of the hypoglossal nucleus in the margin of a plaque in the medulla. Note the heavy microglial reaction and the perivascular cuffing of a venule. Cresyl violet; ×240.



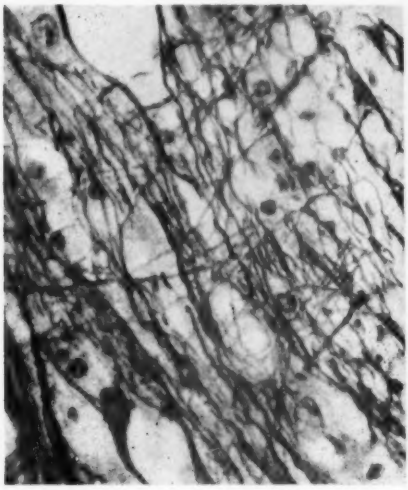


Fig. 3.—Preserved but swollen axons inside a plaque of demyelination of the pons. Bodian stain; × 480.

vation of the neurons in the areas of demyelination was noted throughout. In the marginal zones of the punched-out plaques in the medulla, the nerve cells of the hypoglossal nuclei were still well preserved (Fig. 2). Deeper within the plaques, degeneration and necrosis of nerve cells were apparent, but even in large plaques there was no reduction of axons, although swelling and early fragmentation were sometimes seen (Fig. 3). These axis-cylinder changes were particularly pronounced in the plaque involving the fibers of the left fifth nerve. Early breakdown of the myelin sheaths was seen around blood vessels showing fatty degenerative changes (Fig. 4). Such a relationship of the demyelination of blood vessels was occasionally still recognizable in larger confluent, but sharply circumscribed, demyelinated plaques (Fig. 5).

Glial Tissue

The astrocytes presented considerable alterations. Inside the demyelinated areas they were greatly swollen, and frequently contained two or more nuclei. Bizarre mitotic figures were occasionally seen in these swollen cells, which were devoid of processes (Fig. 7). Marked clasmatodendrosis of globoid astrocytes was also present in a zone immediately surrounding the plaques. Still more to the periphery, proliferated fibrillary astrocytes were seen. Swollen astrocytes with loss of processes were also present in the perivascular areas where the myelin breakdown had just started. The oligodendroglial cells were absent in the demyelinated areas, and a questionable re-

Fig. 4.—Fatty degeneration of the wall of a large venule with early break-down of myelin in the surrounding white matter. Cone and Penfield's silver carbonate method and scarlet red; × 240.

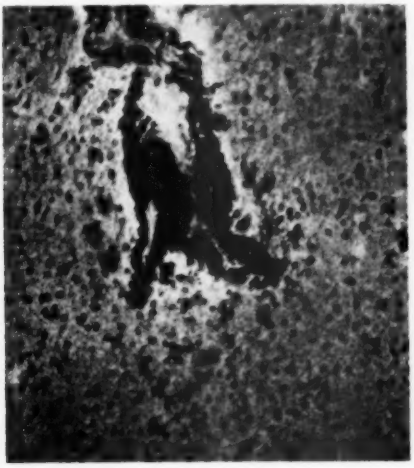
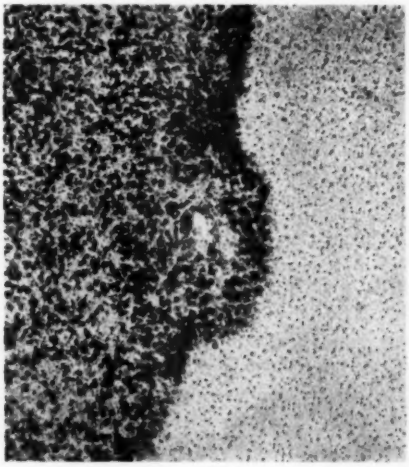


Fig. 5.—Sharply demarcated plaque of demyelination in the left temporal white matter, showing its relation to a blood vessel. Cone and Penfield's silver carbonate method and scarlet red; × 80.



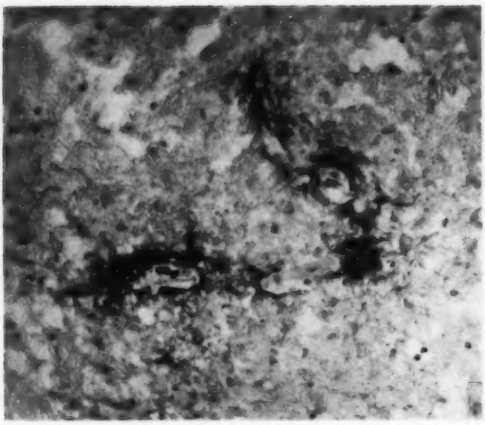


Fig. 6.—Fibrinoid necrosis of arterioles and fibrin threads in the surrounding demyelinated white matter of the pons. Phosphotungstic acid hematoxylin; × 173.

duction in number was noted in the early lesions. The microglial cells were most numerous at the margins of the plaques, where they formed a distinct wall. Most of them were swollen and transformed into gitter cells. These foamy macrophages were unusually large and bizarre in most of the plaques. The nuclei of these cells were sometimes swollen and vesicular. Fusion into large multinucleated, foamy giant cells occurred occasionally.

Mesenchymal Tissue

The walls of the arterioles, capillaries, and venules within and surrounding the plaques were edematous. The endothelial cells were swollen. Hyaline thickening of the walls was rare. Fatty degeneration of the media and adventitial cells was noted in one lesion (Fig. 4). Occasional fibrinoid necrosis with exudation of fibrin into the surrounding tissue was apparent (Fig. 6). Fibrin threads and perivascular hemorrhage were frequently encountered in demyelinated areas, particularly in the medulla. Lymphocytes, plasma cells, and large mononuclear cells were numerous around the vessels within and surrounding the plaques (Fig. 7). The abundance of plasma cells was striking. The walls of the larger veins were infiltrated with lymphocytes distant from demyelinated plaques. Slight increase of reticulin fibers was apparent around vessels which were heavily cuffed by mononuclear cells, mainly inside the demyelinated areas.

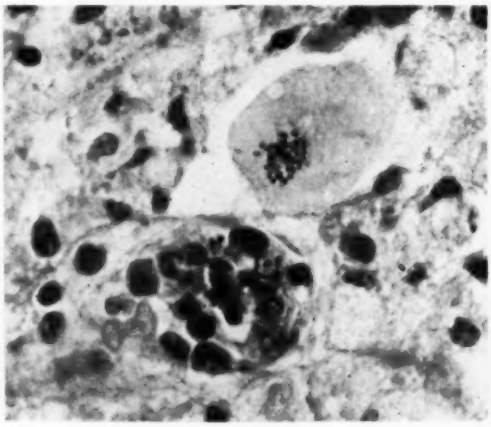


Fig. 7.—Atypical mitotic figure of a greatly swollen globoid astrocyte in the margin of a plaque in the left temporal white matter. Note the numerous plasma cells. Hematoxylin and eosin; × 840.

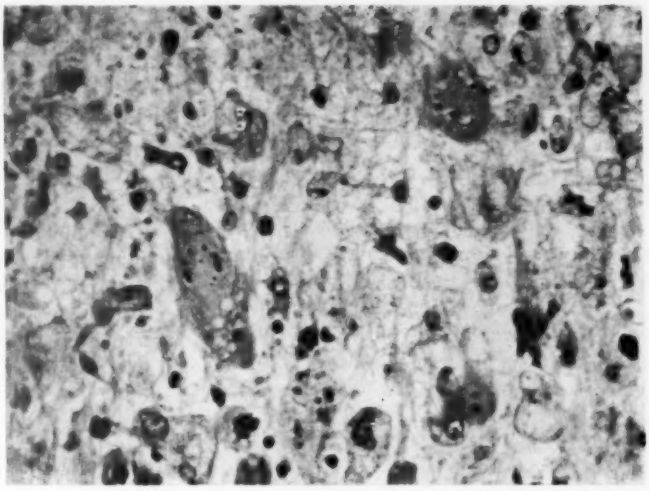


Fig. 8.—Typical cellularity in the margin of a demylinated plaque. Note the giant cells with the numerous peripheral nuclei, the large gitter cells, globoid astrocytes, and scattered plasma cells and lymphocytes. Cresyl violet; × 480.

Giant cells were found in most of the plaques (Fig. 8). They contained numerous small vesicular nuclei, usually situated at the periphery of the cell. Sometimes only dust-like chromatin fragments, remnants of atypical mitoses, were present in the large cells. The cytoplasm of these cells was either foamy or homogeneously pinkish, suggesting that not all of these large cells represented giant gitter cells, but that some of them might have been derived from astrocytes.

The choroid plexus and the pituitary gland were normal.

Comment

This woman's symptoms occurred nine weeks after termination of a four-week course of Co^{60} γ-radiation to the left ear. A total of 5,760 r was given, with daily doses of 288 r. Boden [10] reported seven cases of delayed "radiation myelitis of the brain stem," which presented the first symptoms between 11 and 20 months after the radiation. The total brain-stem doses varied from 4,500 to 6,050 r in the individual cases. More important than the total dose was the time over which the daily doses were spread. The safe dose of radiation delivered in 17 days, as proposed by Boden, ranges from 3,500 r, for large fields, to 4,500 r, for small fields (of the order of a 50 sq. cm. area). These doses are biologically equivalent to from 3,900 to 5,000 r in a period of 28 days when delivered with conventional 250 kv. x-rays. From these figures, correlated by use of Strandquist's biological equivalence curves, it is quite evident that the biological effect of a given dose of radiation can only be evaluated if the over-all time of the course of radiotherapy is known.

In the reported case the highest dose of radiation in brain tissue was 5,170 r (90% of maximal given dose), given in 29 days. This is slightly higher than the figures given by Boden. However, the relative biological efficiency of Co^{60} is considered to be lower than 250 kv. by a factor of 0.85. If this factor is used to correct the dose, then the maximal brain dose becomes 4,400 r.

The lesions consisted of disseminated plaques of demyelination with central necrosis and occasional petechial hemorrhages. These areas were strictly within the beam of Co^{60} and more numerous closer to the source of the radiation. They were well circumscribed, and a central blood vessel was occasionally identifiable (Figs. 4 and 5). Astrocytic proliferation and clasmatodendrosis were marked in and immediately adjacent to the plaques. A wall of microg-

lial cells constituted the margin of the demyelinated areas. The vascular changes were marked but indicated an acute, rather than a chronic, degenerative process. Collagenous hyaline thickening of the arterioles, a common late morphological change following irradiation, was rare. However, the walls of the arterioles, capillaries, and venules were very edematous, and occasionally fatty and fibrinoid changes were noted (Figs. 4 and 5). Perivascular cuffing with plasma cells and lymphocytes was marked.

The localization of the demyelinated plaques within the beam of Co^{60} is strong evidence of an etiologic relationship. The pathogenesis is, however, less evident, for our case does not fit in with what is currently accepted as the effects of radiation on brain tissue.

The first explanation to be considered is the possibility of the lesions being due to vascular damage. Experimental studies on animals have shown that vascular alterations, such as fibrinoid necrosis, may occur approximately three months after irradiation, as in our case (Russell et al.[12] and Scholz [13]). The latent period has been explained by a primary damage to the genetic apparatus of the endothelial cells or fibroblasts, which would become manifest only at the time of reproduction. According to Puck et al.,[14] cells with genetic damage can multiply for even two to five generations before reproduction ceases. The demyelination, however, cannot be explained on an ischemic basis, secondary to these vascular alterations. The preservation of the nerve cells and the axons are very much against such an explanation.

Apart from a radiation damage to vessels, one could speculate on a delayed effect upon the astrocytes and oligodendrocytes. Degeneration of the latter would certainly result in demyelination. Acute oligodendromyelin necrosis has been described following high doses of cathode rays, as mentioned above, but so far we are not aware of publications advancing a delayed radiation effect upon the oligodendrocytes, using x-rays of the order given in our case. The absence of oligodendrocytes in the areas of demyelination does not help in assessing the pathogenesis, because it might be either primary or secondary to the demyelination. Boyesen and Campbell [15] claim that the astrocytes in the vicinity of Pd^{109} and Y^{90} implants are most sensitive to radiation, but this view is not generally accepted.

Proliferation of astrocytes, including multinucleated giant forms with atypical mitotic figures, as in our case, has been noted in many acute demyelinating diseases and has been considered an unspecific finding secondary to severe demyelination. Peters [16] has discussed and illustrated the various bizarre shapes of the proliferated macroglia in acute multiple sclerosis. The resemblance of the punched-out areas of demyelination in our case to the lesions of acute multiple sclerosis is certainly striking. The distribution of the plaques in the subependymal layer and in the gray and white matter, as well as the preservation of nerve cells and axons in the demyelinated areas, is common to both. Although most of our lesions seem to be of the same age, no arrest of the demyelinating process is apparent. A peripheral wall of microglial cells, proliferated astrocytes, and fragments of broken-down myelin indicates a progression of the plaque similar to that seen in acute disseminated sclerosis. The vascular alterations described, however, are not the usual picture, and many authors would also object to the severe inflammatory reaction, as well as to the vascular relationship of the plaques. But if we accept the theory that disseminated sclerosis represents an allergic reaction of the brain, a correlation is perhaps more evident. In experimental allergic encephalomyelitis, Lumsden [17] has stressed the intense histiocytic cuffing of the vessels, and Good [18] has demonstrated the appearance of plasma cells. Ferraro [19] suggests that the giant cells are another feature favoring an allergic pathogenesis of the demyelinating diseases. The abundance of plasma cells, histiocytes, and lymphocytes in the perivascular spaces was particularly

striking in our case, as was the presence of giant cells in the plaques.

To account for the localization of the lesions within the beam of Co^{60}, one must assume that an antigenic substance responsible for such an allergic reaction was produced by the radiation, which might have damaged the oligodendromyelin complex and its enzyme systems, resulting in the accumulation of abnormal metabolites. Such antigens could then stimulate the local accumulation of plasma cells, with production of antibodies, or it could raise the antibodies in the systemic circulation. An antigen-antibody reaction could then take place, either without a preceding destruction of the blood-brain barrier or only after the vessels have become permeable to antibodies at sites of minor vascular damage. In the latter case, the delayed effect of radiation upon the vessels might then play the part of determining the localization of such a reaction. In this respect, it is of interest to note that amyloid has been found in the brain localized to areas of irradiation (Fischer and Holfelder,[6] Lowenberg-Scharenberg and Bassett[20]). It was deposited in the degenerated brain, in the perivascular spaces, and in the vessel walls. The nature of the amyloid is not yet elucidated, but one of the old theories suggests that amyloid represents the precipitate of proteins active in an antigen-antibody reaction (Letterer[21]). We suggest, therefore, that the radiation might have produced a primary alteration of the myelin sheaths, with the release of an antigenic substance. The histopathology of the demyelination is then viewed as an expression of an autoimmune reaction.

Summary

A case of delayed demyelination and necrosis of the brain following Co^{60} irradiation is presented.

The disseminated lesions were limited to the brain tissue within the beam of Co^{60}.

The lesions could not be explained on an ischemic basis secondary to degenerative and obliterative changes of the blood vessels.

The possibility of an autoimmune reaction localized to the irradiated brain tissue is discussed.

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Elastosis in Fibrotic and Cirrhotic Processes of the Liver

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Hohenemser [1] in 1893 described a peculiar increase of elastic fibers in cirrhotic livers. During the next few years this finding was confirmed by several authors, who speculated as to the origin of the newly formed elastic tissue without arriving at definite conclusions.[2-4] For several decades subsequently, this observation found little attention other than brief mention in textbooks and monographs.[5-8] Lately, Gillman et al.[9] noted that fibers staining with Weigert's elastica method may be abundant in "old avascular scars" of cirrhotic livers. They maintained, however, that this change represented degenerated collagen fibers and not an increase of "true" elastica.

The present paper reports histological observations on a series of human livers affected by different fibrosing processes. The presence and the nature of elastica hyperplasia has been studied in relation both to the elastic fibers present in the normal liver and to the possibility of histological differentiation of fibroses of different origins.

Material

Liver tissue from 52 autopsies was studied. The material comprised four groups.

1. Eight normal livers of children, aged 8 months to 10 years, and twelve livers of adults dying of accidents or diseases unrelated to the liver.

2. Five cases of fibrotic and cirrhotic processes of the liver in infants.

3. Fibrotic processes in the liver of adults without widespread transformation of the lobules (17 cases). This group included cardiac fibrosis (5 cases), schistosomiasis (2 cases), biliary cirrhosis (4 cases), subsiding and healed viral hepatitis (2 cases), and early portal cirrhosis (4 cases).

4. Miscellaneous types of cirrhosis of the liver, characterized by severe fibrosis and complete *Umbau* of the parenchyma (10 cases). This group included cases of portal and postnecrotic cirrhosis in various stages of development and one case of hepatolenticular degeneration.

Methods

Most of the material was fixed in Zenker's fluid with acetic acid; some in 10% formalin. The material was collected over a long period of time, and not primarily for histochemical study. On the other hand, the material is standard in that the tissues were all freshly fixed at the time of autopsy and embedded in paraffin within 24 hours. Each staining procedure was carried out for the entire batch simultaneously to allow for peculiarities of the staining solutions, and the methods are summarized in the accompanying Table. Preparations stained with Weigert's resorcin-fuchsin method were taken as a reference point for further evaluation of the nature of elastic structures.

Findings

A. Elastic Fibers Identified by Weigert's Stain

1. *Normal Livers.*—In the capsule of the livers at all age groups, layers of thin elastic fibers were found. In the small portal areas no elastic fibers were present in the children's livers; and the adults often showed short, thin, fragmented fibers, in addition to those of the blood vessels. In the medium-sized and larger portal areas fragments or whole fibers were present in the wall of the bile ducts and a few thin segments in the fibrous tissue around bile ducts and blood vessels.

In the livers of adults who died of diseases not involving the liver, a network of

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Staining Reactions of Elastic Fibers in Blood Vessel Walls and Connective Tissue

Stain	Elastic Fibers in Blood Vessel Wall	Individual Fibers in Connective Tissue	"Confluent Patches" in Connective Tissue
Weigert's	Blue-black	Blue-black	Blue-black
Orcein	Dark brown	Dark brown	Dark brown
Gomori's aldehyde fuchsin	Dark red-purple	Dark red-purple	Dark red-purple
Orcinol-new fuchsin	Gray-black	Gray-black	Gray-black
Hematoxylin and eosin	Pale red-pink	As connective tissue	Sometimes a slight bluish-grayish tinge
Heidenhain's "azan"	Deep blue (in larger arteries sometimes a yellowish core)	As connective tissue	Light sky-blue
Mallory's phosphotungstic acid hematoxylin	Blue-purple (in larger arteries sometimes an orange core)	Coarser fibers blue-purple	Homogeneously pale orange or unstained
Periodic acid-Schiff	Pink-red	As connective tissue	Pale pink
Ninhydrin-Schiff	Pink-red	As connective tissue	Pale pink
Alcian blue-Chlorantine fast red	Unstained or pale pink core with bluish-green borders	As connective tissue	Pale blue tinge
Toluidine blue	Unstained or pale blue	As connective tissue	As connective tissue
Rinehart-Abul Haj	Yellow	As connective tissue	Pale yellow

short, thin elastic fibers was seen surrounding individual collagenous fibers in the portal areas in cases with some fibrotic enlargement of the portal areas.

2. *Fibrotic and Cirrhotic Processes of the Liver in Infants.*—A moderate increase of elastic fibers in the form of short, thin fragments, sometimes arranged in slender bundles, was seen in some of the larger portal spaces and their fibrous extensions in two cases. One was a case of early biliary cirrhosis due to congenital atresia of the extrahepatic bile duct (3-month-old infant), and the second, a case of posthepatitic cirrhosis with localized postnecrotic collapse in an infant who died at the age of 7 months.

No elastic fibers, or only a few thin fibers, were found in the livers of three infants having, respectively, moderate cirrhosis, congenital fibrosis of the liver,[10] and giant-cell hepatitis with diffuse fibrosis.

3. *Fibrotic Processes of the Liver in Adults.*—In 15 of the 17 cases the *capsule* showed a mild increase of elastic fibers with no characteristic qualitative change. However, in four cases of early portal cirrhosis, particularly in cardiac fibrosis, the elastic fibers were severely swollen and often formed small confluent, intensively stained patches (Fig. 1).

In the *portal areas*, which were enlarged by a varying amount of fibrous tissue, an

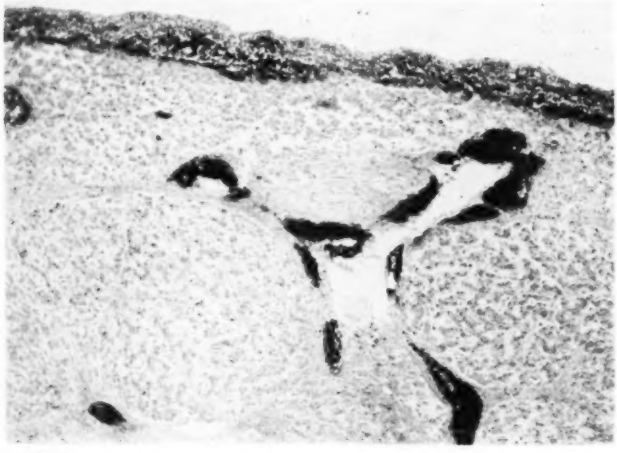

Fig. 1.—Cardiac fibrosis; woman, aged 60 years. In the capsule an increased amount of thickened elastic fibers, forming in some places small elongated patches. Large confluent patches present in the thickened wall of the sublobular and central vein. Orcein stain; × 85.

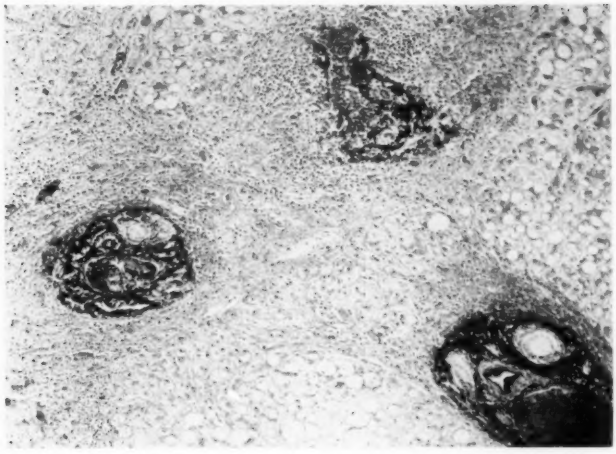

Fig. 2.—Early portal cirrhosis; man, 65 years. Many thickened, densely arranged elastic fibers present in the original portal spaces close to artery, vein, and bile ducts, but absent in the enlarged and heavily infiltrated area. Orcein stain; × 90.

increase of elastic fibers was present. In some places these took the form of short, fragmented, sometimes beaded or nodularly thickened fibers. Elastic fibers were entirely absent from areas of heavy inflammatory infiltration. In cases of postnecrotic fibrosis and early portal cirrhosis elastic fibers were seen in the portal areas only close to the artery, vein, and bile duct (Fig. 2). In the pericholangitic group the elastic fibers were sometimes densely and concentrically arranged around many thickened and fibrosed bile ducts, but the walls of the bile ducts were devoid of fibers or contained only a few short, thin ones.

In the *fibrous extensions* of the portal areas and in the fibrous centrolobular connections in the cardiac group elastic fibers were absent, with few exceptions, which conformed with the occurrence of elastic fibers in the portal tracts proper. Here there were few straight, thin, or slightly thickened elastic fibers, sometimes arranged in narrow bundles (Fig. 3). Rarely, small swollen, confluent patches were present (Fig. 4).

The walls of the *systemic veins* were occasionally thickened, with focal or diffuse increase of swollen elastic fibers, sometimes in the form of elongated, short, confluent

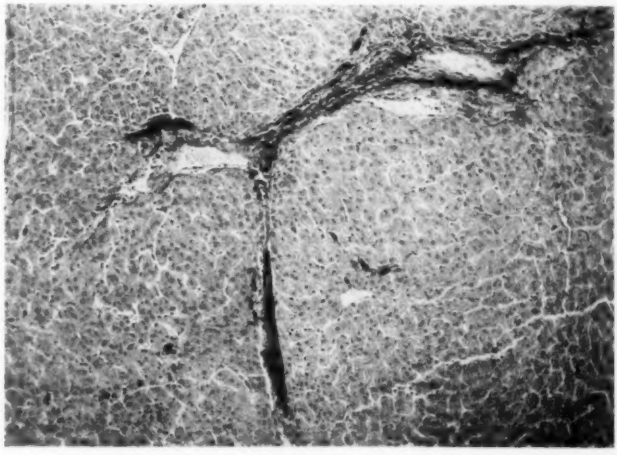

Fig. 3.—Fibrosis of liver in schistosomiasis; man, 45 years. Slender bundles of straight elastic fibers present in the fibrous extensions. Weigert's resorcin-fuchsin (elastica) stain; × 100.

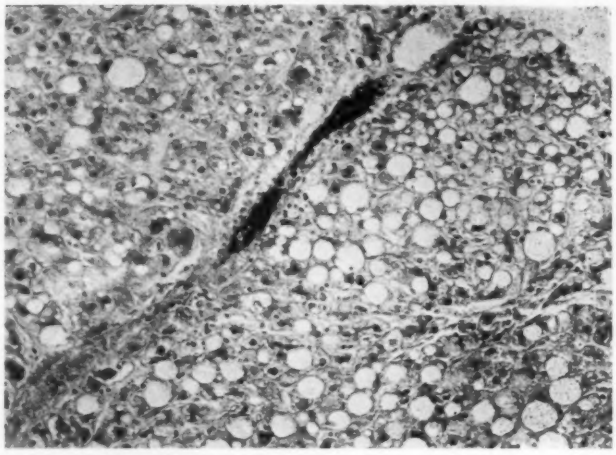

Fig. 4.—Early portal cirrhosis; man, 65 years. Swollen elastic fibers forming elongated confluent patches in a fibrous band. Orcein stain; × 140.

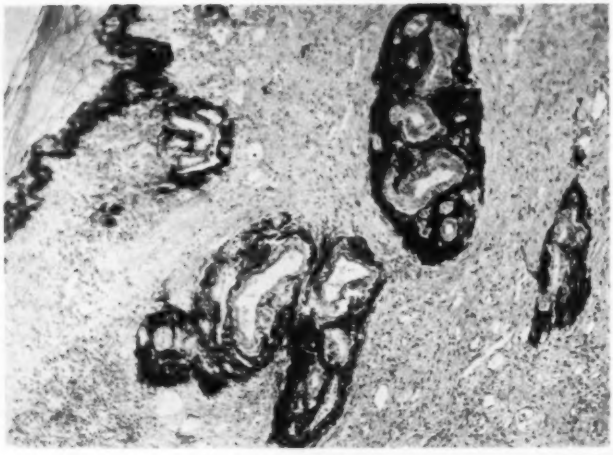

Fig. 5.—Chronic biliary obstruction with localized subcapsular postnecrotic fibrosis; woman, 64 years. Confluent patches of swollen elastic fibers in the capsule and filling the whole portal spaces. The fibrous area itself is infiltrated by inflamelastic fibers. Weigert's resorcin-fuchsin stain; × 100.
matory cells, but is devoid of

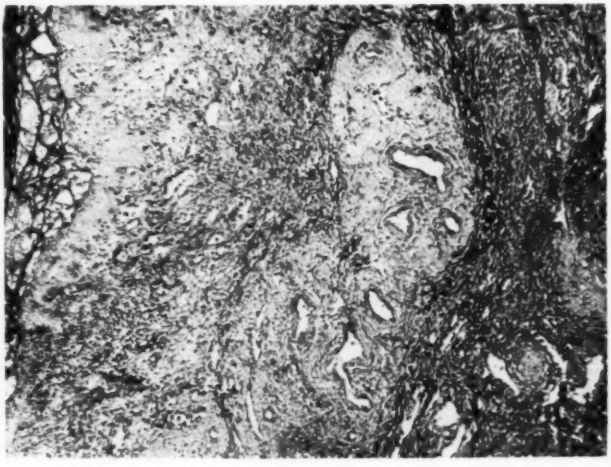

Fig. 6.—Same case as Figure 5. The areas of the confluent patches reveal an homogeneous, almost unstained structure, clearly demarcated from the surrounding fibrillary connective tissue. Mallory's phosphotungstic acid hematoxylin; × 100.

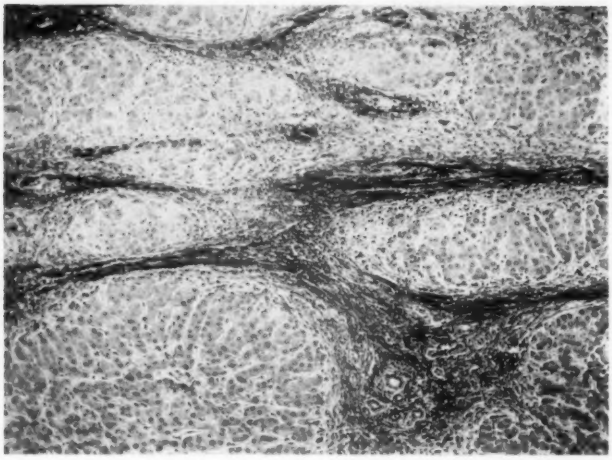

Fig. 7.—Postnecrotic cirrhosis; youth, 14 years. Large amount of elastic fibers arranged in slender bundles, present in the internodular fibrous bands and portal areas. Weigert's resorcin-fuchsin stain; × 85.

patches. Such patches were observed particularly in the cardiac group both in the wall of the sublobular veins and at the periphery of the thickened wall of the central veins (Fig. 1). Round or oval, intensively stained, confluent patches were seen in areas of fibrotic central veins, partly or completely obliterated, particularly in the subcapsular region.

In one case of chronic biliary obstruction the enlarged portal spaces present in an area of postnecrotic fibrosis contained abundant, swollen, confluent elastic fibers (Figs. 5 and 6). The fibrous area itself showed patchy lymphocytic and leukocytic infiltration, with a few bundles of thin, irregularly dispersed elastic fibers.

4. *Cirrhosis of the Liver in Adults.*—In the *capsule,* in areas of subcapsular fibrosis, and in the *internodular* fibrous septa there were many elastic fibers of varying thickness and density (Figs. 7, 8, and 9). In many cases elastic fibers were swollen and fused, transforming the fibrous tissue in places into confluent masses (Fig. 10). Often the wall of the *portal veins* was replaced by swollen elastic fibers, which merged with the elastic fibers of the surrounding fibrous tissue (Fig. 10). At other sites, however, the elastic fibers of the portal

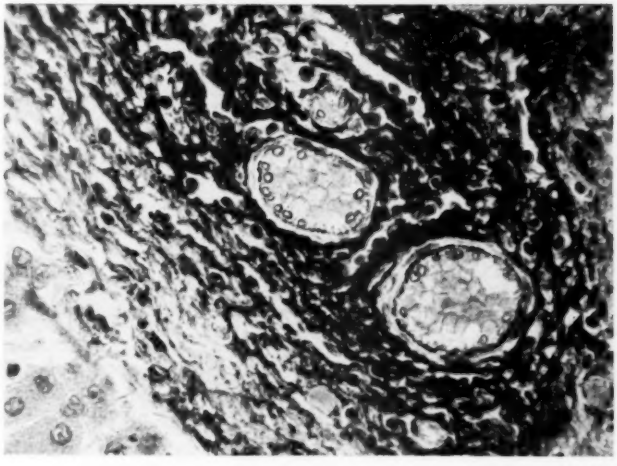

Fig. 8.—Same case as Figure 7. Many irregularly swollen and fragmented elastic fibers in a portal space surrounding small blood vessels. Weigert's resorcin-fuchsin stain; × 510.

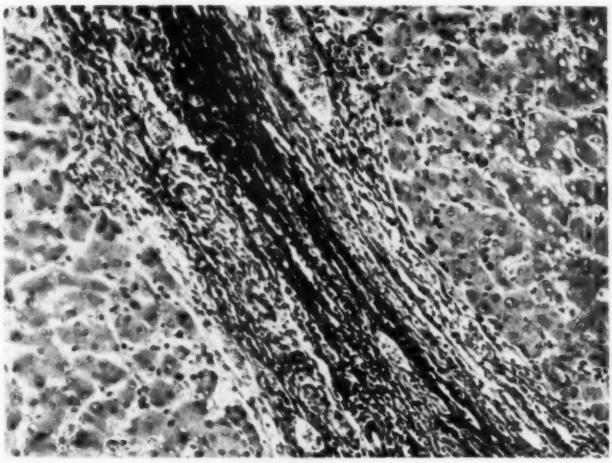

Fig. 9.—Portal cirrhosis; woman, 45 years. Thin and thickened elastic fibers filling almost completely an internodular fibrous band. Aldehyde fuchsin; × 140.

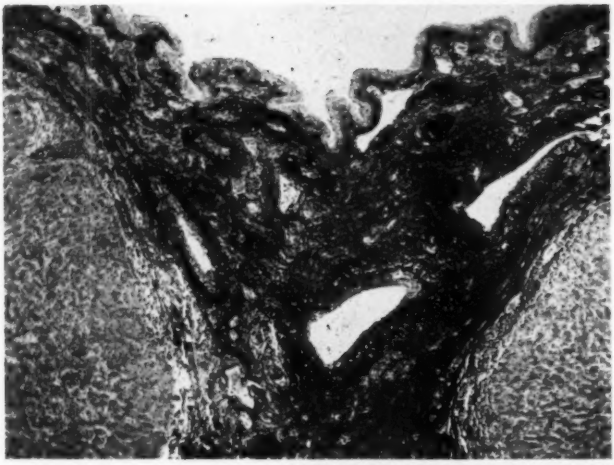

Fig. 10.—Same case as in Figure 9. Capsule, subcapsular area, and broad fibrous band completely filled by thickened and confluent masses of elastic fibers. The elastic fibers present in the wall of the veins and of the connective tissue merge together without a clear border. Aldehyde fuchsin; × 42.

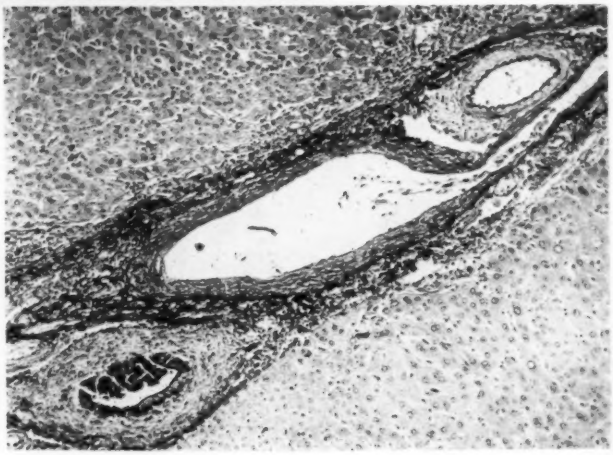

Fig. 11.—Portal cirrhosis, man, 39 years. In the portal space the elastic fibers in the wall of the vein are thin and concentrically arranged, whereas those of the surrounding connective tissue are coarser and by-pass the blood vessels and bile duct Aldehyde fuchsin; × 85.

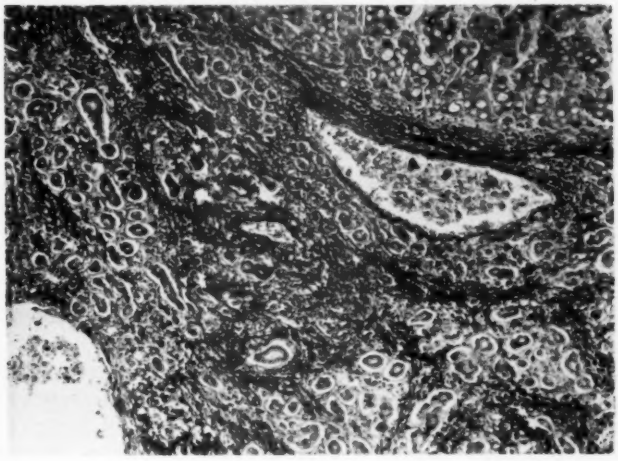

Fig. 12.—Cirrhosis in Wilson's disease; youth, 20 years. Small groups of proliferated bile ducts are enclosed by an irregular network of elastic fibers of various thickness. Aldehyde fuchsin; × 100.

veins and of the surrounding portal spaces could easily be differentiated (Fig. 11).

Single bile ducts or small groups of ducts were surrounded by several layers of wavy elastic fibers (Fig. 12), which were sometimes swollen and confluent. They were generally sparse or entirely absent within the walls of larger or thickened bile ducts, precisely as in the normal liver.

B. Further Staining Reactions of Fibers Identified by Weigert's Method

1. *"Specific Stains" for Elastic Tissue* (Orcein method of Unna-Taenzer, Gomori's aldehyde fuchsin, orcinol-new fuchsin [11]).

All three methods stained nearly the same fibers as those with Weigert's stain. There were differences, however, in the intensity of staining and the differentiation of individual fibers. Elastic fibers within connective tissue septa appeared slightly thicker with Gomori's aldehyde fuchsin stain and more delicate with orcinol-new fuchsin than with other stains. The "confluent patches" appeared always as compact masses, in each case colored identically with the arterial elastic membranes.

2. *Connective Tissue Stains* (Heidenhain's "Azan," Mallory's phosphotungstic acid hematoxylin).—Elastic fibers in blood vessel walls stained clearly with these methods, but individual fibers within connective tissues were not clearly differentiated. The "confluent patches" appeared as homogeneous islands surrounded by fibrillary differentiated collagen tissue. With the "Azan" method the patches colored light blue, sharply defined against the darker-blue surrounding collagen. With Mallory's phosphotungstic acid hematoxylin the patches were unstained or pale orange and were distinct from the surrounding, reddish-brown collagen fibers (Fig. 6).

3. *Hematoxylin-Eosin Stain.*—Elastic fibers in the connective tissue could not be recognized, but the "confluent patches" sometimes showed a blue-gray tinge.

4. *Histochemical Methods.*—(*a*) Periodic acid-Schiff Procedure: Elastic fibers of the blood vessels stained pink-red, but individual fibers in connective tissue could not be differentiated. The confluent patches, however, appeared as homogeneous pale-pink islands within fibrillary connective tissue of the same or a deeper pink color.

(*b*) Ninhydrin-Schiff Method [12]: The coloration and structure were similar to those observed with the periodic acid-Schiff method.

(*c*) Alcian Blue-Chlorantine-Fast Red (Lison [13]): The elastic fibers of the arteries were either unstained or showed a pale pink core, with bluish-green, incomplete borders. The confluent patches were

distinguished by their homogeneous pale-blue tinge from the slightly darker surrounding fibrillary tissue.

(*d*) Aqueous or Alcoholic Toluidine Blue and Rinehart-Abul Haj Colloidal Iron Method: These methods were of no further help in recognition of elastic tissue. The confluent patches stained pale yellow with the Van Gieson counterstain.

Comment

Fibrils staining with Weigert's elastica method were found to be only mildly increased in cirrhotic processes in infants. They were more developed in fibrotic processes of the adult liver, and most of all in the fibrous bands of livers with cirrhosis. In addition, in certain livers, patches of amorphous material were present which stained intensely with Weigert's elastica method.

Is an increase of elastic fibers in various tissues, including the liver, in fact a new formation of true elastic fibers or merely a change in staining qualities of collagen fibers? This problem has been discussed for decades, mainly in regard to "basophilic degeneration" of the skin and to the increase of elastica in arteriosclerotic vessels.[9,14,15] The affinity for elastic stains of degenerated dermal tissue has been variously interpreted as evidence of transformation of collagen to elastic fibers, modification of the ultrastructure,[16-18] or merely the result of more superficial chemical change, as may be produced in vitro.[19-21]

Gillman et al.[9] suggested that morphological differences might help in the differentiation of "true" elastic fibers and collagen fibers with elastotic degeneration. The latter, with acquired affinity for resorcin-fuchsin, were described as coarser, beaded, and fragmented.[9] Such structural characteristics were indeed observed in our cases of cirrhosis, but frequently coexisted with fine elastic fibrils, as seen in healthy tissue. However, the normally great variability in the appearance of elastic fibrils in different tissues of the body is well recognized, as is the occasional presence of "atypical fibrils." Thus, such structural variations as are seen in the light microscope are insufficient for the differential diagnosis of "true elastica."[16,22,23]

This conclusion is further supported by the different staining results, each by itself of doubtful specificity, but in combination useful, in the differentiation of degenerated collagen and elastic fibers.[9] In the fibrosed portions of the liver, resorcin-fuchsin, orcein, and paraldehyde fuchsin stained structures identically with the preformed elastic fibers in the blood vessels and capsule. The same was true of the orcinol-new fuchsin method, which, in the opinion of Fullmer and Lillie,[11] reveals only true elastic fibers. Judgment must be reserved on the amorphous "confluent patches" observed in cirrhoses, and occasionally in the wall of hepatic veins in chronic passive congestion. These areas stained with elastic stains like true elastica, but showed no fibrillary structure. In addition, the "patches" appeared yellow with the Van Gieson stain, palely blue with Heidenhain's "azan" method, and unstained or palely orange with Mallory's phosphotungstic acid hematoxylin, features considered by Gillman et al.[9] to be an indication of "elastotic degeneration" of collagen.

Despite certain differences in staining reactions, our findings support the concept of Gillman et al.[9] regarding the "confluent patches" as "elastotically degenerated collagen fibers." However, the histologic staining methods employed permit the inference of new formation of "true" elastic fibers within the sites of hepatic fibrosis, side by side with "elastotic degeneration of collagen."

It has been maintained that the increase in elastic tissue in the liver is a hyperplasia of fibers normally present, derived particularly from those of the hepatic artery, the bile ducts, and the portal veins.[1,2,4,24] In the present series most of the diffuse elastosis appeared without any topographic relationship to the normal elastic structures. In-

crease of elastic fibers in the hepatic arteries was related to the age of the patient and the degree of general atherosclerosis. Likewise, the thickened bile-duct walls, sometimes present, were largely devoid of elastic fibers. In advanced cases of cirrhosis the confluent elastic fibers of the venous wall sometimes could not be defined from the bundles of elastic fibers of the surrounding fibrous tissue. In areas of less advanced elastosis, the elastic fibers outside could easily be differentiated from those within the venous walls by their independent course, by-passing the vessel, and by the different density and thickness of the interwoven fiber bundles. It is extremely unlikely, therefore, that the elastic tissue of the arteries, the veins, or the bile ducts is the origin of the new elastic fibers.

Whereas areas of inflammation and young granulation tissue showed little or no elastosis, elastic fibers appeared gradually in areas of formation of cellular fibrillary connective tissue. They developed strikingly when the fibrous tissue became older, anuclear, and hyalinized. Thus, in general, the increase of elastic fibers appeared related to the quality and amount of the newly formed connective tissue, irrespective of the type of fibrosis and cirrhosis of the liver. This eliminates the possibility of using elastic tissue as a point of differentiation between "true Laennec's cirrhosis" and other types of hepatic cirrhosis.[24]

In tissue repair with scarring and in keloids, elastic fibers have rarely been observed,[25,26] the liver being one of the few exceptions.[5,6] Among other factors [6] tensile and shearing effects in the cirrhotic liver were suggested to be of importance for the developing elastosis,[5] and a number of points may be enumerated in favor of this assumption. In the cirrhotic liver the regenerated nodules exert pressure on the portovenous circulation, and obviously also on the surrounding bands of connective tissue.[27] The severe elastosis in the hepatic veins in cases of congestive fibrosis may conceivably be related to increased hemostatic pressure. Depending on the degree of pressure, perhaps both "true" elastic fibers and "elastotic degeneration of collagen" are increased; this accounts for the prevalence of "true" elastic fibers in the fibrotic livers and the prevalence of "elastotically degenerated" ones in the cirrhotic livers, where the pressure may reach the highest degrees.

Some support of the mechanical hypothesis is seen in the increased amount of elastic fibers in the endocardium in cases of mitral stenosis.[28] Mechanical factors have also been proposed as important in the induction of fibrous tissue in fatty livers [29] and in fatty metamorphosis and massive necrosis.[30] On the other hand, absence of elastic fibers has been demonstrated in all degrees of experimental cirrhosis produced by carbon tetrachloride [31] or by nutritional methods.[32]

The unique position of the human liver with regard to new formation of elastic fibers requires further clarification, which cannot be obtained by analogies but might be expected from studies with morphologic-enzymatic methods, such as have been applied to investigations of dermal elastosis [19-21] and by studies of the ultrastructure.[16-18,33]

Summary

The presence and characteristics of elastic fiber were investigated in 32 livers with various fibrotic and cirrhotic processes and in 20 normal livers of children and adults.

In fibrotic livers stained with "special stains" for elastic tissue, the amount of elastic fibers was found to be greatly increased. This increase was observed in the liver capsule, the portal spaces, fibrous bands, and the walls of the portal and systemic veins. Two different types of elastosis were observed: 1. Elastic fibers of various structure and thickness, straight or undulated, and forming irregular networks or bundles of changing diameters. In their staining properties, the fibers corresponded to "true" elastic fibers, as seen in the healthy dermis and arteries. 2. In various processes, particularly in cases of

cardiac fibrosis and in cirrhosis of all types, swollen elastic fibers were observed, which fused together to form "confluent patches" of various shapes and sizes. These patches stained very strongly with the usual elastic stains and were homogeneously chromophobic in Mallory's phosphotungstic acid hematoxylin. Thus, these patches possessed the qualities reported in the literature for elastotic degeneration of collagen.

The increase of elastic fibers appeared related to the quality and amount of the newly formed connective tissue, being most prominent in areas of acellular, hyalinized connective tissue and least, or absent, in areas built by cellular fibrillary young connective tissue and in areas of inflammation. It appeared that the histologic study of elastic fibers is of little significance in the differential diagnosis of fibrotic lesions of the liver.

The hypotheses concerning the causes of elastosis in the diseased liver are discussed with special regard to the effect of mechanical stresses.

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Growth of Human Epidermoid Carcinoma in Tissue Culture Using Nonfat Milk Medium

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A cell-culture maintenance medium containing autoclaved nonfat milk has been described by Baron and Low.[1] The viral sensitivities of cell cultures in this medium were generally greater than those of cultures in a maintenance medium containing calf serum, and serum antibodies added to the nonfat milk were completely inactivated when it was autoclaved in the manner used for sterilization. Baron and Low suggested that the milk medium might be of value in the isolation of viral agents not previously cultured because of serum antibodies or nonspecific serum viral inhibitors in maintenance media containing serum.

During studies with a strain of phagocytic cells derived from a murine malignant lymphoma (Strain $P388_{D1}$), the malignant cells were first grown in a medium containing 40% human serum and then transferred to a maintenance medium composed of 20% autoclaved nonfat milk and 80% mixture No. 199.[2] After prolonged maintenance in this serum-free medium, adaptation of the cell strain to growth in the medium was observed.[3] Subsequently, this cell strain has been carried in the nonfat-milk medium for one and one-half years and has been used for studies in vitro with the polyoma virus.[4]

After it had been observed that the nonfat-milk medium supported the growth of the murine lymphoma cells in culture, attempts were made to isolate a cell strain from a human malignant tumor in the same medium. This report describes a strain of cells (NCLP-1) from a human epidermoid carcinoma, grown in a serum-free medium containing 20% autoclaved nonfat milk. The cell strain has now been grown in culture for one year and has been carried through four consecutive passages with preservation of structural characteristics of epidermoid differentiation.

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Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

Materials and Methods

Medium.—The medium used in these studies consisted of 20% autoclaved nonfat milk and 80% mixture No. 199. The methods used in preparation of this medium have been described previously.[3]

Source and Preparation of Tissue.—The tissue was obtained from a 49-year-old white man who was admitted to the Clinical Center of the National Institutes of Health in December, 1957, because of an ulcerated lesion on the chin of seven months' duration. A biopsy specimen was obtained, and the histopathologic diagnosis was epidermoid carcinoma of the skin of the chin. On Jan. 6, 1958, the lesion was resected, and bilateral submandibular lymph node dissections were performed. Microscopically, the tumor was a fairly well-differentiated epidermoid carcinoma with keratinization, epithelial pearls, and intercellular bridges (Fig. 1). Metastatic carcinoma was found in one lymph node from the right side of the neck.

Tumor tissue for culture studies was obtained approximately 10 minutes after the surgical specimen was resected. Using aseptic technique, tissue which was grossly identified as carcinoma was removed from the primary lesion. An attempt was made to select tumor tissue with as little adherent stroma as possible. The tumor tissue was then placed in a Petri dish with 1 ml. of milk medium and minced with iris knives. The fragments were distributed on the glass surface of a sterile 2 oz. Sani-Glas prescription bottle and allowed to adhere to this surface for approximately 30 minutes at 36 C. One milliliter of the milk medium was then added to the bottle, and the culture was again incubated at 36 C. Additional medium was added on the following day, and the volume in the bottle was brought to 5 ml. The

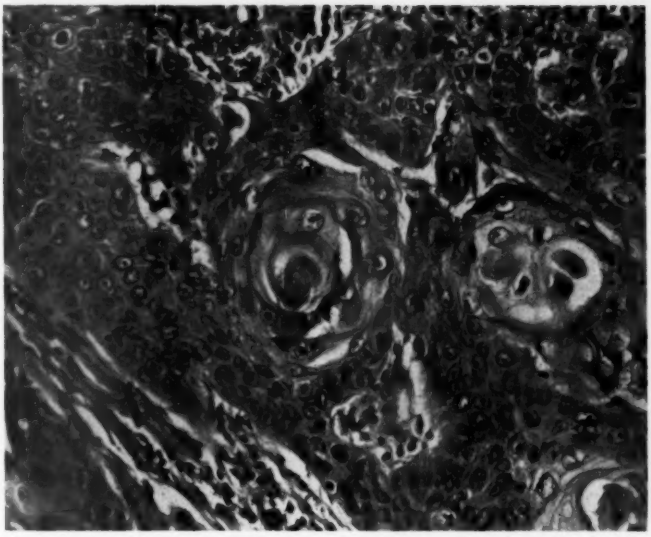

Fig. 1.—Section of primary epidermoid carcinoma of skin of chin of a 49-year-old man. Keratinization and pearl formation can be seen. Hematoxylin and eosin; reduced to 83% of mag. × 270.

culture was fed every two to three days, depending upon changes in pH of the medium.

Methods of Subculture.—Subcultures from established bottle cultures were carried out by scraping cells from the glass surface with a Pasteur pipette and transferring them to a new bottle. Prior to subculture, 4 ml. of medium was removed from the bottle, and approximately one-fourth of the area of the culture was scraped from the glass surface with the tip of a pipette. The cellular material was dispersed in the remaining 1 ml. of medium, and this was transferred to a new bottle. The clumps of cellular material were distributed on the surface of the new bottle, and approximately one-half of the medium (0.5 ml.) was removed. The new bottle was then incubated at 36 C for two to three hours. One milliliter of milk medium was then added to the bottle, and it was incubated at 36 C. Additional medium was added on the following day, care being taken not to dislodge the adherent clumps of cells from the glass surface. The volume of fluid in the bottle was gradually increased until there was 5 ml., and feeding was carried out every three to four days, depending upon pH changes.

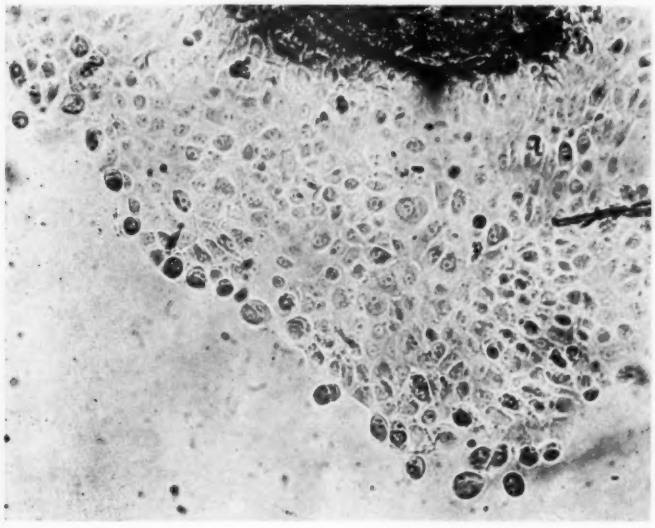

Fig. 2.—Initial culture of cell strain NCLP-1, derived from a human epidermoid carcinoma, 30 days after explantation. A sheet of epithelial cells is seen growing from the margin of an explant. Unstained; reduced to 92% of mag. × 95.

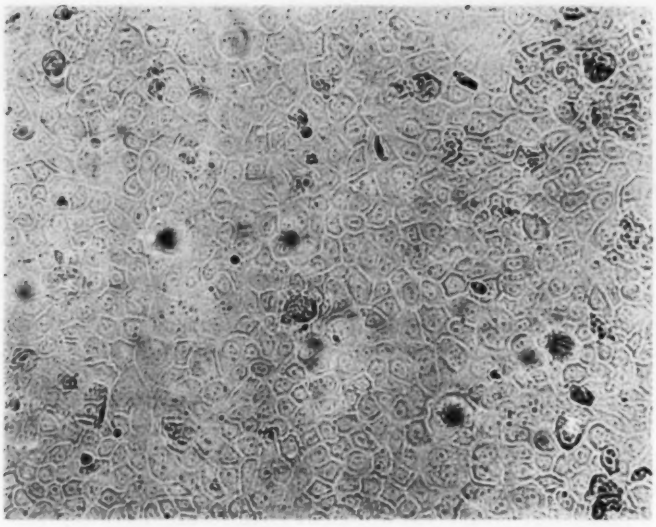

Fig. 3.—Cover-slip culture of cell strain NCLP-1, 329 days after initial culture was started and 154 days after transfer of clumps of cells from initial culture to cover slip in Leighton tube. A confluent sheet of polyhedral cells is seen. Unstained; reduced to 88% of mag. × 140.

Results

On the ninth day after the bottle culture had been initiated, epithelial cells were first seen migrating from the periphery of some of the explants. One month after the culture had been started, small sheets of epithelial cells had grown from many of the explants (Fig. 2). After two months, large sheets of epithelial cells had grown from almost all of the explants. No fibroblast-like cells were seen at any time in the culture. There was considerable nuclear pleomorphism, and collections of cells resembling epithelial pearls were found. In some areas the growths from several explants met to produce large confluent sheets of epithelial cells, covering most of the dependent surface of the bottle.

On the 58th day, a portion of the culture was scraped from the glass surface with a

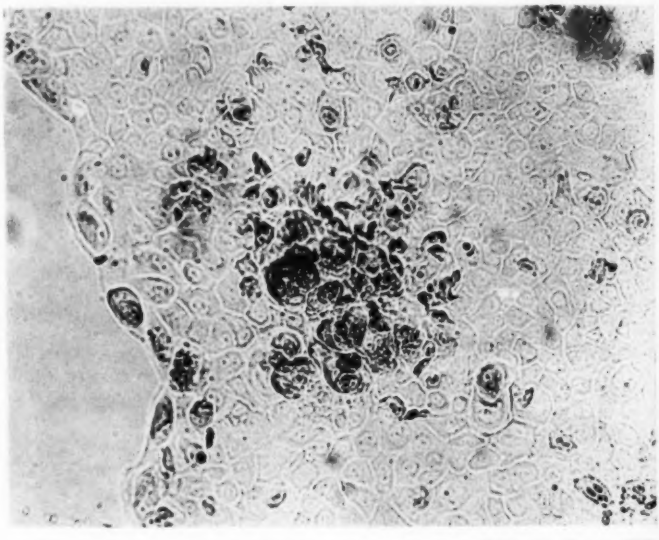

Fig. 4.—Culture of cell strain NCLP-1 (same culture as in Figure 3). Note epithelial-pearl formation. Unstained; reduced to 88% of mag. × 140.

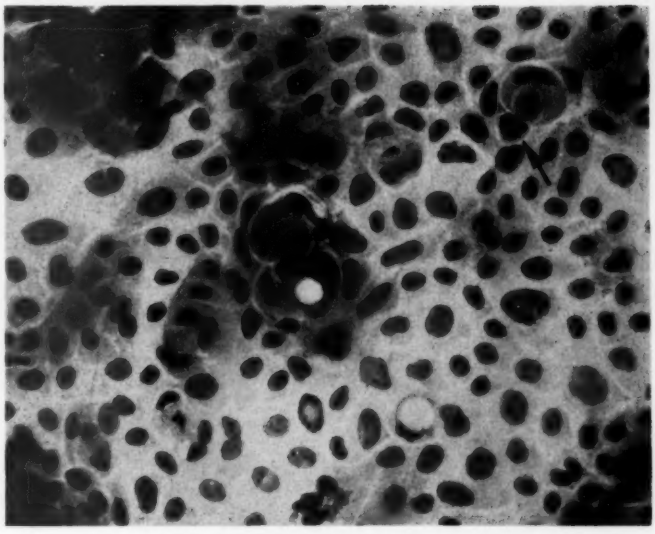

Fig. 5.—Cover-slip culture of cell strain NCLP-1, 301 days after initial culture was started and 189 days after transfer of clumps of cells from the initial culture to cover slip in Leighton tube. Note nuclear pleomorphism, keratinized cells (indicated by arrows), and epithelial-pearl formation. Ether-alcohol fixation, Papanicolaou stain; reduced to 83% of mag. × 270.

pipette and transferred to a new bottle. Clumps of cellular material adhered to the glass of the new bottle, but no outgrowth of cells was seen during the subsequent 16 days. Another transfer was attempted on the 69th day, and this was also unsuccessful. On the 84th day, using the procedure described above, a successful transfer was accomplished. Clumps of cells adhered to the glass surface of the new bottle, and, after one week, sheets of cells were seen migrating from the periphery of the clumps in a manner similar to that seen with the explants in the original culture. No growth was seen from scattered single cells which had adhered to the surface. After several weeks the cell sheets became confluent and covered the entire surface of the bottle.

Material from bottle cultures has been transferred to cover slips in Leighton tubes, and large confluent sheets of epithelial cells have been grown on the cover slips (Fig.

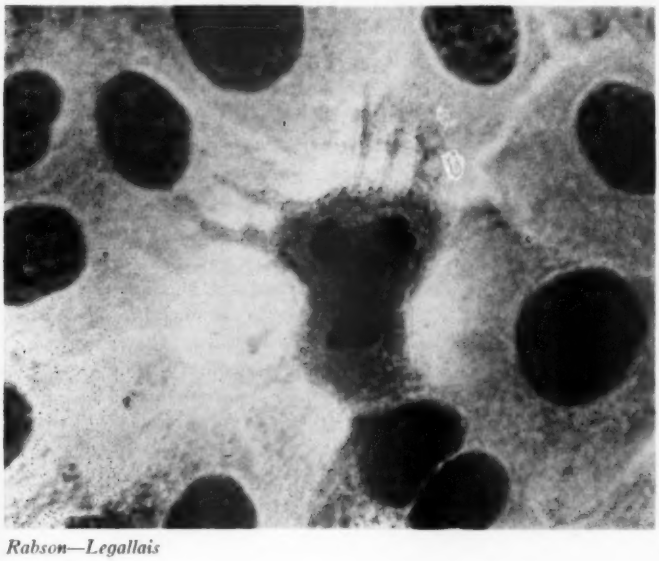

Fig. 6.—Culture of cell strain NCLP-1 (same culture as in Figure 5). Note tripolar mitosis. Coarse chromatin granules and prominent nucleoli can be seen in nuclei of adjacent cells. Ether-alcohol fixation, Papanicolaou stain; reduced to 83% of mag. × 1,350.

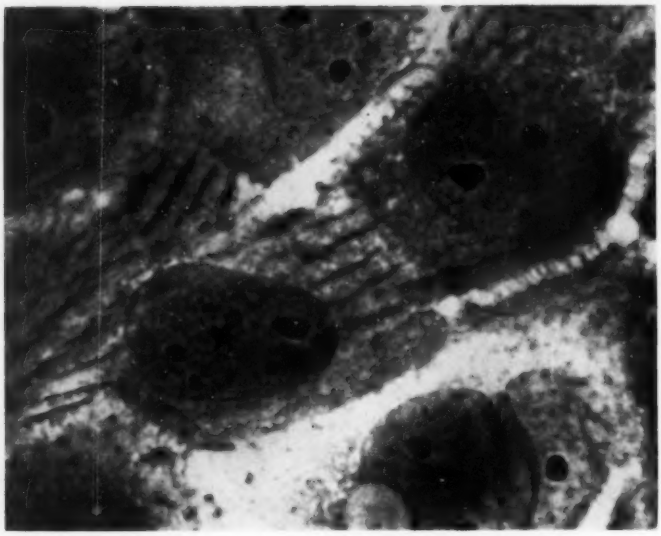

Fig. 7.—Cover-slip culture of cell strain NCLP-1, 330 days after initial culture was started and 201 days after transfer of clumps of cells from the initial culture to cover slip in Leighton tube. Note tonofibrils and intercellular bridges. Fixation in Zenker's solution with 10% formalin, stained with Heidenhain's iron-alum hematoxylin; reduced to 83% of mag. × 1,700.

3). Clusters of cells arranged in epithelial-pearl formations can be seen in these cultures (Fig. 4).

Subsequently, it has been possible to start five new bottle cultures from the original bottle, and the cell strain has been carried through four consecutive subcultures during a period of one year. Twenty-three cover-slip cultures have been grown in Leighton tubes for fixation and staining.

In fixed cover-slip cultures stained with the Papanicolaou stain, keratinized cells and epithelial pearls are seen (Fig. 5). The cells have pleomorphic hyperchromatic nuclei and prominent nucleoli, and abnormal mitotic figures are present (Fig. 6). In preparations fixed in Zenker-formol solution and stained with Heidenhain's iron-alum-hematoxylin method, tonofibrils and intercellular bridges are clearly identifiable (Fig. 7).

Comment

There have been many reports of growth of human epidermoid carcinomas in short-term tissue culture, and in some of these epithelial-pearl formation and other structural characteristics of epidermoid differentiation have been described.[5,6] Several cell strains have been established from human epidermoid carcinoma tissue and have been kept in culture for long periods [7-9]; however, no reports were found of keratinization, epithelial-pearl formation, or intercellular bridges in such cultures. The cells of the strain described in the present paper grow slowly and are more difficult to subculture than the rapidly proliferating, undifferentiated epithelial-cell strains. The fact that epidermoid characteristics are maintained, however, is of interest, and may be of some advantage in attempts to isolate viruses from such diseases as molluscum contagiosum, laryngeal papillomatosis, and cutaneous warts, in which viral etiology is suspected but from which no agents have as yet been isolated in culture.

Growth in a medium free of serum should also be advantageous in virus isolation studies. Since the nonfat milk is autoclaved before use, the introduction of extraneous viruses in the medium is less likely than in systems in which cells are grown in serum-containing medium. Other advantages of avoiding whole serum in the medium in virus studies have been described by Chang and Geyer,[10] and the relatively full virus sensitivity of cell cultures in a maintenance medium containing autoclaved non-

fat milk has been discussed by Baron and Low.[1]

The epidermoid carcinoma from which this cell strain was derived was the first human tumor we attempted to grow in the autoclaved nonfat milk medium. Subsequently, we have tried to grow six other human tumors, without success. These were two choriocarcinomas, a malignant melanoma, a chondrosarcoma, a mesothelioma of the pericardium, and an adenocarcinoma of the ovary. As yet no further attempt to grow other human epidermoid carcinomas in this medium has been made. In view of the cytotoxic action of normal human serum on some lines of atypical human cells in vitro,[11] the use of serum-free milk medium in such studies seems indicated.

Summary

The isolation of a cell strain from a human epidermoid carcinoma of the skin in a serum-free medium containing 20% autoclaved nonfat milk is described. The strain has been grown in vitro for one year and has been carried through four consecutive passages with preservation of structural characteristics of epidermoid differentiation. Keratinization, epithelial-pearl formation, tonofibrils, and intercellular bridges have been demonstrated in the cultures.

The authors would like to express their gratitude to Mr. Gebhard Gsell and Mr. John McGuire, who prepared the photomicrographs, and to Mr. Frank Miner for technical assistance.

Pathologic Anatomy Branch, National Cancer Institute (14).

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Congenital Hemiplegia Resulting from Cerebral Malformation

Terminal Complication of Myeloid Leukemia

E. CLARENCE RICE, M.D., and ANATOLE DEKABAN, M.D., Washington, D. C.

The pathologic lesions underlying hemiplegia dating from birth are multifold. The commonest causes include birth injury and prenatal destructive lesions. Although malformation of the brain is a relatively frequent finding in children with cerebral palsy, unilateral involvement is very rare. The patient to be reported exhibited a localized agyria limited to the anterior half of the left cerebral hemisphere. The remainder of the brain was well formed save for the ipsilateral corticopontile and corticospinal tracts, which were deficient as a result of the malformed cortex. The interest in this unfortunate child is further increased by the occurrence of an extensive hemorrhagic infarction of the normal hemisphere, this being related to myeloid leukemia, which finally led to the death of the patient.

Report of a Case

A 10-year-old girl. Apparently the pregnancy was normal, and delivery took place at term. The precise details of the birth are unknown, although it has been suggested that there may have been an unspecified birth injury. During the first days of life the infant was not very vigorous, and her subsequent development was retarded. From the age of 9 weeks she began to have frequent cerebral seizures, which were characterized by stiffening of all extremities and upward deviation of the eyes. Neurological examination at 3 months of age disclosed right hemiparesis and left esotropia. On various occasions it was noticed that a proportion of her attacks were focal and consisted of jerky movements in the right extremities. She was given phenobarbital, and the attacks decreased in number. At the age of 7 months she was found to be chronically constipated, and the left side of her abdomen was bulging. A barium enema revealed megacolon. She had several short-lasting hospitalizations for treatment of constipation, convulsions, and other minor ailments. At the age of 18 months she began to take a few steps, although with a limp on the right side. She pronounced her first word when 2 years old. The patient was investigated at 7 years of age because of increased difficulty in bowel movements and a poor general condition. At that time it was noted that the right side of her body was smaller than the left, and the presence of right hemiparesis was confirmed. Funduscopic examination under general anesthesia revealed an abnormally small left optic disk. The electroencephalogram showed a slow-wave focus in the left frontoparietal region. An unexpected finding consisted of a high count of white blood cells, which ranged from 33,000 to 58,000 per cubic millimeter on various examinations, with large numbers of immature cells of the myeloid type. Bone-marrow biopsy showed pronounced myeloid hyperplasia and a decreased number of erythroid cells. The findings were interpreted as diagnostic of chronic myelocytic leukemia. She was treated by x-irradiation of the spleen and subsequently with oral busulfan (Myleran) and followed up in the blood dyscrasia clinic of Children's Hospital. During the subsequent three years of her life the white blood cells ranged from over 200,000 to 7,000 cells per cubic millimeter. Between the ages of 3 and 7 years she was seen and treated in the cerebral palsy clinic for right hemiparesis and epilepsy. Both these conditions were attributed to possible birth injury. Since 6 years of age she had been enrolled in a special school for handicapped children, where she made slight progress. During her final hospitalization she was greatly emaciated and had a high fever most of the time. The white cell count was elevated and the number of blood platelets greatly reduced. She exhibited a bleeding tendency in the form of gross hematuria, melena, and epistaxis

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From the Children's Hospital, and the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

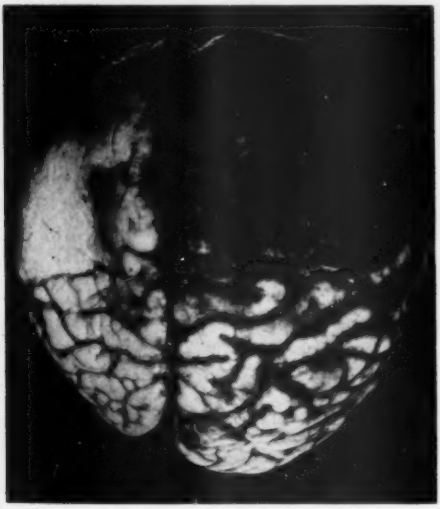

Figure 1

Fig. 1.—Small left cerebral hemisphere with agyria. Subarachnoid hemorrhage involving right and left hemispheres.

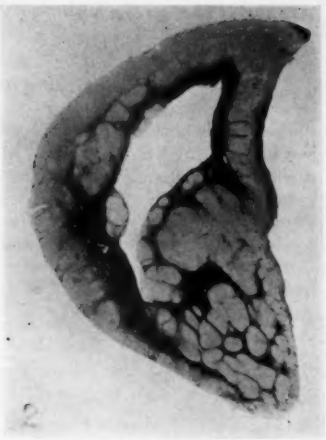

Figure 2

Fig. 2.—Large left lateral ventricle.

Eventually uncontrollable convulsions developed; she became comatose and died.

Postmortem examination disclosed (1) chronic myelogenous leukemia, (2) multiple hemorrhagic ulcerations of the skin and viscera, (3) infarction of the anterior half of the right cerebral hemisphere, (4) congenital malformation of the anterior half of the left cerebral hemisphere, and (5) megacolon and absence of the myenteric ganglion.

The left cerebral hemisphere was smaller than the right, and the surface of the anterior half was lacking in normal convolutions, its surface being smooth or slightly granular (Fig. 1). There was a small area of localized subarachnoid hemorrhage over the left frontal pole and extensive hemorrhagic infarction of the anterior part of the right hemisphere, with considerable effusion of the blood into the subarachnoid space over the con-

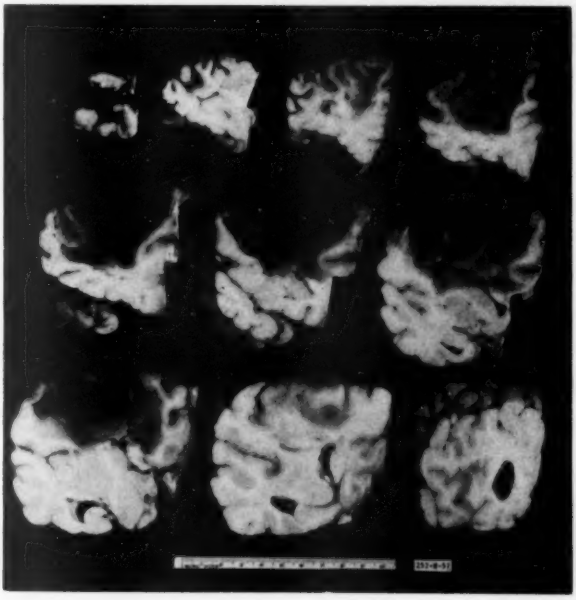

Fig. 3.—Infarction of right cerebral hemisphere.

vexity of this hemisphere (Figs. 1 and 3). The basal and lateral parts of the brain were free from hemorrhage. The brain was sectioned transversely. The left hemisphere anteriorly showed a large ventricular cavity which decreased to the normal lumen in the posterior parietal region. The right lateral ventricle was of normal size. The brain parenchyma of the right hemisphere showed a large area of hemorrhagic infarction, which extended from the anterior frontal region to the central convolution. The right cerebellar hemisphere was slightly smaller than the left. The left pyramid was missing.

Histological examination confirmed hemorrhagic infarction of the right hemisphere and showed agyria of the anterior part of the left hemisphere, with numerous areas of heterotopia of the immature neural elements extending from the lateral ventricle to the cortex (Fig. 2). The cortex in this region was very wide and was populated by elongated neuroblasts, which were arranged in a haphazard way, without any tendency toward lamination. The posterior part of the left hemisphere showed normal cortical architectonics.

Comment

Lack of differentiation of the cerebral pallium into cortical convolutions is called agyria, or lissencephaly. This is one of the most primitive malformations of the cerebral cortex and is generally considered to be a result of arrested development of the brain before the fetus has reached 14 weeks of gestation. The estimation of the earliest possible teratogenic period is difficult, although it is unlikely that a noxious factor acting as early as the first six weeks of prenatal life could lead to this anomaly. In the majority of the cases reported, the agyria was bilateral.[1-5]

Absence of the ganglion cells in the myenteric plexus is commonly associated with megacolon. Since the primitive neuroblasts in the peripheral ganglia are already present by the 6th week of prenatal life and the cortical malformation in the form of agyria occurs between 6 and 14 weeks of gestation, these two developmental lesions must have occurred at different stages of prenatal life. This is true only if the absence of ganglion cells in the autonomic ganglia is a result of agenesis, and not of their destruction. We have no clue regarding the possible etiology of disturbed development in this patient. The family history and the course of pregnancy were said to be normal.

It is of interest that the right hemiparesis and also the epileptic attacks, were attributed to a suspected birth trauma, and that the possibility of unilateral congenital malformation of the brain was not considered at all. The hemorrhagic infarction of the anterior part of the right hemisphere was predominantly confined to the pool of the major branches of the middle cerebral artery. The most probable mechanism of vascular obstruction was thrombosis, to which the child was predisposed by the increased viscosity of blood and slowing of general circulation related to dehydration.

It has been noted that the children suffering from congenital malformations have a slightly higher risk of developing leukemia than their normal mates. Krivit and Good [6] estimated that the occurrence of leukemia in children with mongolism was about three times as high as that which might be anticipated from chance association.

Summary

The case is reported of a child who had suffered from cerebral palsy since birth, and who at the age of 7 years developed myeloid leukemia, which finally led her to death. Postmortem examination disclosed the following findings: agyria of the anterior part of the left cerebral hemisphere; absence of ganglion cells in the myenteric ganglion, associated with megacolon; recent hemorrhagic infarction of the anterior half of the right cerebral hemisphere, and myeloid leukemia. The teratogenic period and pathologic findings are discussed.

Children's Hospital, 2125 13th St., N. W. (9).

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News and Comment

SOCIETY NEWS

The annual business meeting of the American Society for Experimental Pathology was held April 14, 1959, in Convention Hall, Atlantic City, with 70 members present.

DECEASED MEMBERS

Glenn H. Algire
Nathan Chandler Foot
Stephen S. Hudack
Arthur Kirschbaum

MEMBERSHIP STATUS

533	Active members
14	Retired (Federation Proceedings)
20	Retired recently
567	

CURRENT REQUESTS FOR RETIREMENT STATUS (10)

Arnold R. Rich
Robert A. Lambert
Eric L. Alling
Frank L. Apperly
James P. Simonds
E. T. Bell
Lloyd L. Arnold
Ernest W. Goodpasture
A. L. Bloomfield
Claude S. Beck

NEW MEMBERS (44)

Alfred A. Angrist
John Palfrey Ayer
William J. Cheatham
William Christopherson
Kelly H. Clifton
Arthur I. Cohen
Robert D. Coye
Gustave J. Dammin
Arthur M. Dannenberg Jr.
Franklin G. Ebaugh Jr.
Marilyn G. Farquhar
Martin H. Flax
Adam James French
Gabriel C. Godman
John D. Hartman
Henrik A. Hartmann
Thomas R. Harwood
Cecil Hougie
Dwight J. Ingle
George L. Jordan
Donald W. King Jr.
Robert R. Kohn
Irwin H. Lepow
Anton Lindner
Richard A. MacDonald
Robert T. McCluskey
Frank T. Maher
Hans Meier
Jurgen R. Meyer-Arendt
Robert W. Mowry
John J. Murphy
Gordon B. Pierce
Robert E. Priest
William E. Ribelin
Roy Ellot Ritts
Ernesto D. Salgado
Fenton Schaffner
C. J. Shellabarger
Herschel Sidransky
Irving L. Spar
Vernie A. Stembridge
John J. Trentin
Henry E. Weimer
George F. Wilgram

It was approved as presented. The Treasurer's report showed a deficit of $136.03. It was pointed out in the discussion that additional income would be expected from the proration of the increase in registration fees for the annual meeting. The Treasurer's report was unanimously accepted.

Dr. Emory Warner reported for Dr. Harold L. Stewart on the International Organization of Pathology Societies. The desirability of such an organization to consult with U. N. and W. H. O. on matters dealing with pathology was presented. The discussion which followed brought out the continuing interest of the Society members in such an international organization. It was voted to continue in the International Organization by continuing in the Intersociety Committee, in which the four other Pathology Societies were represented.

Dr. Erickson reported on the activities of the Advisory Council. In regard to meeting places, there was some discussion that San Francisco and Chicago might alternate with

Atlantic City. Atlantic City seemed to be the most popular meeting place in the subsequent discussion.

Four dollars ($4.00) of the dues go to the running of the Federation, which is an organization of societies and not an organization of individuals.

The increasing size of the meeting has been a matter of discussion of the Advisory Council. More papers were being presented by more people. There had been discussion of lengthening the meeting to nine days or more. This was felt undesirable by the membership. Evening sessions were not desired by the membership, nor did restriction of presentation of papers every second year, or some such device, including ruling that any one member's name shall appear only once on the presentation, seem desirable.

Dr. F. W. Hartman reported on the activities of the Intersociety Committee for Pathology Information. He pointed out that the Parke-Davis Award and the meeting of the Pathology Society were being covered by P-R Associates, the firm employed by the Intersociety Committee. Dr. Robert B. Jennings reported on the Public Information Committee. Dr. William B. Wartman reported on the A. M. A. ARCHIVES OF PATHOLOGY, which is the official publication of the American Society for Experimental Pathology. It was brought out in the discussion that the Editor of the ARCHIVES was anxious to maintain and to further the association and that the fact of affiliation of the journal with the Pathology Society would be more conspicuously indicated. A member from the floor questioned the value of the relation to the ARCHIVES OF PATHOLOGY and wished reexamination of the affiliation. It was the general feeling that this affiliation would continue to be valuable and should be maintained.

The Histochemical Society meeting in relation to the Federation sessions was discussed and deemed desirable. It was pointed out that next year the Histochemical Society would meet with the anatomists because of some special anniversary meeting of the anatomists. However, in the future the Histochemical Society will continue to meet with the Federation of American Societies for Experimental Biology.

The Officers of the American Society for Experimental Pathology were elected for 1959-1960.

Dr. C. C. Erickson	President
Dr. Jacob Furth	Vice-president
Dr. J. F. A. McManus	Secretary-treasurer
Dr. William B. Wartman	Past-president
Dr. K. M. Brinkhous	Councilor and Assistant Secretary-treasurer
Dr. R. W. Wissler	Councilor

In discussion, it was pointed out from the floor that $8.50 was a very high price to pay for a banquet. The Secretary promised that in the future less expensive banquets would be sought but that this was not exorbitant for Atlantic City and the Shelburne Hotel.

At the banquet which was held on April 15 at the Shelburne Hotel, 106 members and guests were present. The Parke-Davis Award was presented by the President, Dr. William B. Wartman, and by the chairman of the Selection Committee, Dr. Douglas Sprunt, to Dr. Stanfield Rogers for his exceptionally meritorious investigations on tumors. The Parke-Davis Award Lecture was delivered at 9:00 a. m. on April 16, 1959.

There was general discontent with the meeting rooms given to the Pathology Society, and future secretaries will be warned against these spaces. It was agreed that the meeting rooms were an unfortunate combination of poor construction and ventilation, difficult to reach, and isolated.

In the subsequent secretaries' meeting, Cytology was continued as an Intersociety session title, and an attempt will be made to include the Gastrointestinal Tract as the title for an Intersociety session. Next year will see the initiation of mechanical programing, in all probability. A preliminary meeting of the secretaries will be held at the end of June in an attempt to work out the details of such a programing.

In summary, the condition of the Society appears to be good. The membership is increasing satisfactorily, although a more active search for new members will be reinstituted. The present members should consider desirable candidates of their acquaintance and propose membership for them. The financial affairs of the Society are at a bare level of support, but we cannot be sure about this until the report comes in from the Atlantic City meeting concerning the proration of the increase in registration fees. Scientifically, the Society is flourishing.

J. F. A. MCMANUS, M.D.
Secretary-Treasurer

ANNOUNCEMENTS

Fourteenth Annual Symposium on Fundamental Cancer Research.—The 14th Annual Symposium on Fundamental Cancer Research, on the subject "Cell Physiology of Neoplasia," will be held Feb. 25, 26, and 27, 1960, at the University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston.

Symposium on Evaluation of Early Diagnosis of Cancer.—A Symposium on Evaluation of Early Diagnosis of Cancer will be presented at the Annual Scientific Session of the American Cancer Society to be held Oct. 26-27, 1959, at the Biltmore Hotel, New York City. Details as to the program may be obtained from Dr. Scott Hill, Director, Professional Education, 521 W. 57th St., New York 19.

Urology Award.—The American Urological Association offers an annual award of $1,000 (first prize, $500; second prize, $300, and third prize, $200) for essays on the result of some clinical or laboratory research in urology. Competition is limited to urologists who have been graduated not more than 10 years and to hospital interns and residents doing research work in urology.

The first prize essay will appear on the program of the forthcoming meeting of the American Urological Association, to be held at the Palmer House, Chicago, May 16-19, 1960.

For full particulars write the Executive Secretary, William P. Didusch, 1120 N. Charles St., Baltimore. Essays must be in his hands before Dec. 1, 1959.

GENERAL

Dedication of Richard J. Finnegan Memorial Building.—In connection with the dedication ceremonies of the Richard J. Finnegan Memorial Building, established as part of the La Rabida-University of Chicago Institute, the following pathologists contributed to the scientific symposium, on June 13, 1959:

Dr. Paul Klemperer: Role of Connective Tissue in Human Diseases

Dr. Jean Oliver: Past and Present Directions in Renal Research

PERSONAL

Dr. Stanhope Bayne-Jones Recipient of Passano Award.—Dr. Stanhope Bayne-Jones has been selected as the recipient of the $5,000 Passano Award for 1959. The award was made in Atlantic City on June 10, and was in recognition of his many contributions to science and medicine.

Dr. Emmanuel Farber Appointed to American Cancer Society Professorship.—Dr. Emmanuel Farber, of the Department of Pathology at Tulane University School of Medicine, New Orleans, has been appointed to an American Cancer Society professorship in cancer research, the appointment being designed to support a program of lifetime cancer research. This represents the seventh lifetime cancer research professorship which has been established by the American Cancer Society.

Dr. Paul Klemperer Receives Honorary Doctor of Science Degree.—Dr. Paul Klemperer, of the Mount Sinai Hospital, New York, received an honorary Doctor of Science degree at the University of Chicago Spring Convocation on June 12, 1959.

Dr. Robert E. Stowell Appointed Scientific Director of Armed Forces Institute of Pathology.—Dr. Robert E. Stowell, of the University of Kansas School of Medicine, has accepted the appointment as Scientific Director of the Armed Forces Institute of Pathology, succeeding Dr. Ernest W. Goodpasture, who resigned on April 10, 1959. Dr. Stowell has been professor and chairman of the Department of Pathology and Oncology, and director of Cancer Research at the University of Kansas for the past 11 years. For the past three years he has also served as a member of the Scientific Advisory Board of Consultants of the Armed Forces Institute of Pathology.

Books

Our Nuclear Adventure—Its Possibilities and Perils. By D. G. Arnott. Price, $6.00. Pp. 170, with 17 illustrations. Philosophical Library, Inc., 15 E. 40th St., New York 16, 1958.

This excellent monograph surveys both the potentials and the problems of nuclear energy. Fortunately, the author is neither alarmist nor an irrepressible optimist as he discusses life in the atomic age. He starts on the assumption that his reader has little or no knowledge of radiation physics or biology and in the first two chapters presents basic information in both fields in such a clear manner that the chapters are a sheer delight. In the second part, both the theory and the effects of atomic weapons are explained, including megaton weapons. Here, too, the problems of radiostrontium and weather modification from bomb explosions are discussed. While Sr^{90} presents a potential hazard, the author prudently rejects the thesis that test explosions to date have demonstrably changed weather patterns. The real promises of atomic power, both fission and thermonuclear power, are considered in the third portion of the book. Economic development of those countries lacking abundant natural power is now possible, so that utilization of this new-found power holds real promise for mankind.

In the fourth part of the book, the author leaves factual discussion and permits himself to consider the philosophical problems of our age. First, he considers the philosophical and social implications of the nuclear bomb and concludes that "this weapon at least should be immediately renounced" and hopes that the weight of world disapproval will be sufficient for its control. Nuclear power and international control, as well as secrecy and responsibility, are also covered in this section. It is in this philosophical discussion that the book is weakest. The author is an outstanding British authority on atomic sciences; and, while he obviously feels strongly about the moral problems of atomic energy, he does not have the background to present his philosophical arguments in a compelling manner. Nevertheless, he has written a concise, clear, rational volume of the possibilities and perils of the nuclear age.

Year Book of Cancer—1957-1958 Year Book Series. Randolph Lee Clark Jr., B.S., M.D., M.Sc., D.Sc., and Russell W. Cumley, B.A., M.A., Ph.D., Editors. Price, $8.00. Pp. 523, with 191 illustrations. Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1958.

Pathologists have long been acquainted with the purpose and general excellence of the series of Year Books, representing the various medical specialties and published for the past 50 years years by the Year Book Publishers, Inc. In the "Year Book of Cancer" series all fields of medicine are represented as they are applicable in research, diagnosis, or therapy relating to cancer. The editorial board of the current volume consisted of 124 leaders in medicine, centered in the staff of the M. D. Anderson Hospital in Houston, Texas. From the total of 4,000 articles published during the year, this board selected 235 for inclusion in the Year Book. After this selection, the authors of the original articles were asked to prepare abstracts for the "Year Book of Cancer." In this volume 84 journals are represented, the majority being published in the United States.

The volume systematically considers both diagnosis and treatment of tumors of the various organ systems. Following this, specific problems in pathology and cytology, radiotherapy, chemotherapy, and rehabilitation are discussed. The final sections concern epidemiology, chemistry, radiobiology, and genetics in relation to tumors. A concluding article, written specifically for the "Year Book of Cancer" surveys oncology in the Soviet Union. The choice of articles and the preparation of abstracts in this volume are good—a merit which strongly recommends the "Year Book of Cancer" to pathologists not only concerned with diagnosis and treatment of tumors but interested also in some of the more basic problems of tumors.

Blood Groups. By A. E. Mourant, Chairman of Committee of Symposium. Price, $3.25. Pp. 174, with illustrations. Medical Department, The British Council, 65 Davies St., London W. 1, England, 1959.

The 14 topics cover everything important in the field of blood groups: blood group antibodies in man and in seeds, antiglobulin reaction, hemolytic disease of the newborn and

its management, relation to other diseases, chemistry, blood group substances in human tissues and plasma and in animals, blood groups and natural selection, anthropology, genetic linkage, and inheritance. The authors are British leaders, and some are world leaders in the area which they cover. The list of titles is evidence of the phenomenal growth of our knowledge in the field of blood groups and of its constantly increasing application far beyond blood transfusions. The 81 pages contain the last word in the field, well written and remarkably comprehensive, thanks to admirable selection. Well-chosen references are included. An excellent job.

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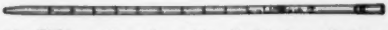

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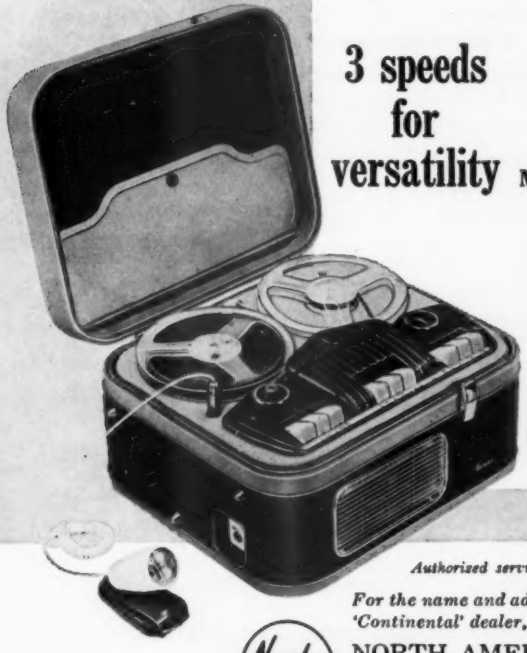

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